

# **IDENTIFICATION OF EXTRACTION METHODS FOR THE PRODUCTION OF HUMIC ACIDS FROM BLACK LIQUOR**

By

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**DECLARATION**

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any University for a Degree.

Signature:.....

Date:.....

**ABSTRACT**

South Africa has a variety of sources for humic substances that may be utilised for the large-scale production of specialised fertilizers. Humic substances are dark coloured fully decomposed remains of plant and animal organic matter. Amongst the sources that were investigated in this study, a 5-weeks old compost (C5W), a 6-weeks old compost (C6W), a compost prepared by Medallion Mushroom farm for the production of mushrooms (CB1), a compost produced as waste material after mushroom production (CB2), recycled water used in compost (i.e. CB1) preparation for mushroom production (“Goodie water,” GW) and black liquor from pulp industry (BL), black liquor showed the best potential for the large scale production of humic acids in South Africa. The quantity and quality of humic acids in these materials were determined by using the basic acid-precipitation method and E6/E6 ratio analyses of the products.

Extracting humic acids from black liquor was optimised by comparing three possible variations to the standard humic acid-precipitation method (HAp), namely, lignin extraction/humic acid precipitation method (LHAp), humic acid precipitation/acid treatment method (HApA) and lignin extraction/humic acid precipitation/acid treatment method (LHApA). The method of extraction had a substantial impact on the quantity and quality of humic acids produced. Humic acid precipitation method followed by humic acid precipitation/acid treatment method (HApA) demonstrated high quantities of humic acids compared to lignin extraction/humic acid precipitation method (LHAp) and lignin extraction/humic acid precipitation/acid treatment method (LHApA).

Analyses such as Fourier Transform Infrared (FTIR) spectroscopy, Ultraviolet Visible Spectroscopy (UV-VIS), High Performance Liquid Chromatography (HPLC) and Elemental Analysis demonstrated that the quality of humic acids produced from black liquor was similar to those reported previously. The type of material (e.g. softwood, hardwood and bagasse) used for black liquor production significantly affected the elemental and molecular composition of humic acids produced. The quality of humic acids produced was affected by both lignin extraction and acid treatment. However, chemical analyses indicated that the features of the humic acids extracted from black liquor correlated with their lignin origin. Humic acids from hardwood and softwood black liquor had a low nitrogen and high sulphur content compared to those extracted from black liquor produced from bagasse material. Although chemical and instrumental analysis of humic acids was quite reasonable for all humic acids produced,

LHAp method was favoured for the extraction of humic acids from pine liquor due to the high quantity of humic acids produced (low S and ash contents).

The humic acids extracted by means of HAp method and HApA had a positive effect on plant growth, when extracted from bagasse and *Eucalyptus* black liquors. Humic acids extracted by HAp from pine liquor had an inhibitory effect on plant growth. A possible process flow for the large-scale production of humic acids from black liquor was formulated. The possible process flow indicated that with the amounts of black liquor produced from pulp industry production of humic acids in South Africa is a possibility. Nevertheless, there is still more that needs to be done to investigate the cost effectiveness of this production prior its implementation. HAp method was most suitable for the production of humic acids from *Eucalyptus* and bagasse liquors in terms of quantity and plant response but LHAp method was more suitable for the production of humic acids from pine liquor in terms of both quantity and quality.

## OPSOMMING

Suid-Afrika het 'n verskeidenheid bronne van humusstowwe wat vir die grootskaalse produksie van kunsmisstowwe gebruik kan word. Humusstowwe is donkergekleurde plantaardige en dierlike organiese materiaal wat ten volle afgebreek is. Van die bronne wat in die studie ondersoek is, is 'n vyfweek-oue kompos (C5W), sesweek-oue kompos (C6W), 'n kompos wat deur Medallion Mushrooms vir sampioenproduksie voorberei is (CB2), gehersirkuleerde water wat tydens komposvoorbereiding vir die produksie van sampioene gebruik word – bekend as “Goodie Water” (GW) – en swart afvalvloeistof van die papier-en-pulp-industrie (BL). Swart afvalvloeistof het die beste potensiaal getoon vir grootskaalse vervaardiging van humussure in Suid-Afrika. Die kwantiteit en kwaliteit van die humiensure in bogenoemde stowwe is deur die suurpresipitasie-isolasiemetode en die E4/E6-verhoudingsanalise van die produkte bepaal.

Ekstrahering van die humiensure vanuit swart afvalvloeistof is geoptimeer deur drie moontlike variasies van die standaardhumienuurpresipitasie-metode (HAp metode) met mekaar te vergelyk, naamlik die lignienekstraksie- of humienuurpresipitasie-metode (LHAp metode), humienuurpresipitasie- of suurbehandlingsmetode (HApA metode) en 'n lignienekstraksie- of humienuurpresipitasie-metode (LHApA metode). Die ekstrasiemetode het 'n beduidende impak gehad op die kwantiteit en kwaliteit van die humienuurproduksie. HAp en HApA metode het groter hoeveelhede humiensure geproduseer in vergelyking met LHAp metode en LHApA.

Analises soos die Fourier Transform Infrarooi Spektroskopie (FTIR), Ultraviolet Visuele Spektroskopie (UV-VIS), Hoë Verrigting Vloeistof Kromatograaf (HVVK) en Elemente Analises het getoon dat die kwaliteit van die humiensure wat van swart afvalvloeistof geproduseer is, soortgelyk was aan dié van voorheengerapporteerde data van ander bronne. Die tipe materiaal (sagtehout, hardehout en bagasse) wat in die produksie van swart afvalvloeistof gebruik is, het 'n beduidende invloed gehad op die element- en molekuleêre samestelling van die humiensure wat geproduseer is. Die kwaliteit van die humiensure wat geproduseer is, is deur die lignienekstraksie sowel as die suurbehandling beïnvloed. Chemiese analises wys egter dat die kenmerke of eienskappe van die humiensure wat uit swart afvalvloeistof geëkstraheer is met hul lignienoorsprong korreleer. Die humiensure van die swart afvalvloeistof wat van hardehout en sagtehout geproduseer is, het 'n lae stikstof- en hoë swawelinhoud gehad in vergelyking met die humiensure van die swart afvalvloeistof wat

van bagasse geproduseer is. Alhoewel die chemiese analises vir al die geproduseerde sure redelik goed was, word HAp metode en HApA verkies weens die hoë kwantiteit tydens produksie.

Die humiensure wat deur HAp metode en HApA geëkstraheer is, het 'n positiewe effek op die groei van mostertsade gehad wanneer geëkstraheer uit swart afvalvloeistof wat van bagasse en *Eucalyptus* geproduseer is. Humiensure wat deur middel van metode A uit denne-afvalvloeistof geëkstraheer is, het 'n inhiberende effek op die plantegroei gehad. Hierdie inligting het gelei tot 'n konsepionele prosesvoorstelling vir die moontlike grootskaalse produksie van humiensure. Die prosesvoorstelling het getoon dat die swart afvalvloeistof wat deur die papier-en-pulp-industrie geproduseer word genoegsaam sal wees om humiensure in Suid-Afrika te produseer. Daar moet egter nog baie navorsing gedoen word om die koste-effektiwiteit van die produksie van humiensure te ondersoek voordat dit geïmplementeer kan word. HAp metode is die effektiëfste manier om humiensure te ekstraheer uit swart afvalvloeistof wat van *Eucalyptus* en bagasse geproduseer is.

## **DEDICATION**

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## GLOSSARY

HAp:	Humic acid precipitation method
LHAp:	Lignin extraction/humic acids precipitation method
HApA:	Humic acid precipitation/acid treatment method
LHApA:	Lignin extraction/humic acid precipitation/acid treatment method
SOM:	Soil organic matter
SOC:	Soil organic carbon
HS:	Humic substances
HA:	Humic acid
FA:	Fulvic acid
C:	Carbon
S:	Sulphur
K:	Potassium
Na:	Sodium
O:	Oxygen
C/N:	Carbon nitrogen ratio
NaCl:	Sodium Chloride (salt)
-COOH:	Carboxyl group
-OCH <sub>3</sub> :	Methoxyl group
-OH:	Hydroxyl; group
C5W:	a compost sample of 5-weeks old compost material
C6W:	a compost sample of 6-weeks old sample material
CB1:	compost prepared by Medallion Mushroom farm for the production of mushrooms.
CB2:	Compost produced as waste material after the production of mushrooms
BL:	black liquor sample collected from Sappi Kraft
GW:	Goodie Water
C_com:	commercial compost obtained from a Agrishop.
Mg:	Magnesium
SO <sub>2</sub> :	Sulphur Dioxide
IHSS:	International Humic Substances Standard
E4/E6:	ratio of absorbance at 465 and 665 nm determined by UV-VIS spec.

VU-VIS spec: Ultraviolet Visible spectroscopy  
FTIR spec.: Fourier Transform Infrared Spectroscopy  
HPLC: High Performance Liquid Chromatography

## **CHAPTER 1. INTRODUCTION**

### **1.1 INTRODUCTION**

Humic substances are extremely versatile and critical components of the natural soil-ecosystem, where they have persisted for hundreds of years (Mayhew, 2004). They are the most widely used distributed organic products of biosynthesis on the surface of the earth, exceeding the amount of organic carbon contained in all living organisms by approximately one order of magnitude (that is, tenfold) (Mayhew, 2004). They provide a concentrated and economical form of organic matter that can replace humus depletion caused by conventional fertilization methods in soil. The addition of humic substances to soils can stimulate plant growth beyond the effects of mineral nutrients alone. Humic substances are extensively used all over the world due to their benefits in agricultural soils, especially in soils with low organic matter. They form an integral part of the ecosystem as they play an important role in global cycling of nutrients and carbon.

In the 20<sup>th</sup> century, Hayes et al. (1985) and Levinsky (2005) demonstrated that it was less expensive to grow plants in mineral nutrient solutions with added humic substances, than using nutrient solutions alone. On the other hand, products containing humic substances provide economically and ecologically friendly solutions by increasing plant nutrients and reducing the use of conventional fertilizers (Tarhan, 2003).

Humic substances derived from coal have been a matter of controversy for over 200 years. The frustration in the development of these agricultural products came from the industrial sector. Based on the promising implications of early research, some companies began production of various humic extracts from low rank coals. Products were in many cases pushed by forceful marketing strategies and with claims of miraculous qualities. However, for the most parts these products did not deliver the claimed results. These developments led to a general mistrust of humic substance soil amendments among the scientific and agricultural community (Kline and Wilson, 1994). Besides their presence in soils, humic substances can be found in varying concentrations in a number of different sources such as rivers, lakes, oceans, composts, sediments, peat bogs and soft coal. They are an incredibly colloidal mixture that has never been separated into pure components. Inconsistent use of terminology and the previous lack of standard materials for comparison purposes have compromised the ability to

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translate the sparse amount of scientific knowledge into practical applications in soil environments.

The term humic substance is a generic name used to describe coloured material or its fractions including humic and fulvic acids. In this study, the hypothesis that black liquor is a potential source for the large-scale production of humic acids is investigated by comparing this material with other materials which are also likely to contain humic substances.

Currently, humic-based products, mainly derived from Leonardite, are increasingly popular in organic farming as well as in outdoor horticulture and greenhouse cropping. Their market is likely to continue growing, provided a few problems such as exaggerated claims as to the miracle effectiveness of these products, are sorted out (Metzger, 2003). Commercial humic-based products are available from several suppliers such as Dayal Fertilizer (Pty) Ltd. in India, Biomechanical in the UK, Humic Tech in Australia, Italy and some other countries. These products are packaged in liquid, powder or granular form (Tarhan, 2003).

In South Africa, agricultural production is done largely on a commercial basis, which by implication involves monocropping and heavy use of inorganic fertilizers. This practice has, in general, been detrimental to soils and the environment. Meanwhile, there is a growing interest in South Africa and many other countries for organic farming, which has necessitated the need to increase production of organic fertilizers. Humic substances are commonly used as organic fertilizers or soil amendments, and energy sources mainly because of their chemical composition and properties.

Although the benefits of organic fertilizers are well known, many farmers are not convinced enough to move away from the use of conventional fertilizers. This could partly be attributed to limited supply of high quality organic fertilizers. Therefore, a lot of work needs to be done if organic fertilizers are to replace conventional fertilizers in South Africa. It is important to consider studies towards finding ways of making organic fertilizers and other organic compounds necessary for farming, available for large-scale application. This study is among few initiatives aimed at providing practical scientific information and technology on how humic acids from humic substances could be extracted in qualities and quantities suitable for large-scale production and application in South Africa.

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In addition it is, in most cases, difficult to get organic nutrients such as humic acids from humic substances in their pure form. Increasing supply of organic compounds, in particular humic acids in their pure form, would require identification of a suitable source for humic substances with substantial amounts of humic acids.

In South Africa the pulp production process produces black liquor, which contains lignin-derived humic substances in large quantities. Mondi Business Paper, Mondi Packaging Felixton and Sappi Saiccor are some of the pulp and paper companies that produce large amount of black liquor that would provide a sustainable source of raw material for the production of humic acids for large-scale production.

Another major limiting factor for the production of high quality humic acids on large scale in South Africa could be associated with the method used for extracting these substances. The basic extraction method i.e. acid precipitation method, currently in use neither discretely removes unwanted material, nor prevents the extracted materials from recombining with free radicals of contaminants. Methods that are currently available for purification of these humic acids are unfortunately too expensive to be considered for large-scale production and quite limited to batch processes. It is therefore important that appropriate extraction methods are established that could allow production of acceptable quality of organic nutrients in particular humic acids, from humic substances more effectively, and efficiently at large scale. Hypothetically, use of a resin material for the purification of humic acids would be considered as the cheapest method, but more investigations need to be considered for its use in large-scale production. For this study, in comparison to the standard method (acid precipitation method) for extraction of humic acids from some selected raw materials this method was adopted and modified. A systematic process with identified control points for extracting humic acids is necessary for developing an effective and efficient extraction method.

It is generally acknowledged scientifically that soil fertility suitable for plant growth is dependent on a combination of factors like concentration of elements in the soil, the amount of applied fertilizers, the type of fertilizers (liquid or solids) and the availability of nutrients when plants require them. These concepts are also relevant for effective use of humic acids as compounds for promoting plant growth. Agriculture, as one of South Africa's most important industries, is likely to benefit from humic acids products because of their contribution to improved micronutrients release efficiency in plants (see Appendix 8.1, Figure 8.1.1) (Allison, 1973). A year-to-year consistency in production rates may be possible with humic-amended

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soils. It is acknowledged in the literature that humic acids have been shown to stimulate both the number and length of roots in young, growing plants (Kononova, 1966). In this study, assessing the effect of humic acids on growth of some South African plant species was necessary.

## 1.2 PROBLEM STATEMENT

In South Africa there is pressure to increase productivity of agricultural systems to meet domestic and international demands. Most of the agricultural systems practice monoculture, which depletes soil nutrients as compared to intercropping systems. This, combined with the high economic costs associated with crop failure, has encouraged widespread use of fertilizers and other agrochemicals. In some parts of the country, demand for agricultural productivity has led to marginalization of farmers, forcing them to cultivate unsustainable soils, in risky climatic areas, in order to produce food. These pressures, especially when combined with poor cultivation techniques such as removal of plant material after harvesting, leads to increased runoff and soil erosion. Use of fertilizers is indeed important to achieve optimum yields. However, it is equally important to fully understand the implications of the type of fertilizer applied in the soil. Excessive use of reduced (energy rich) sources of nitrogen in South Africa has been one of the causes of soil acidification, following its biological oxidation to nitric acid.

It has long been recognized that humic substances have many beneficial effects on soils and consequently on plant growth (Mayhew, 2004). However, since the dawn of mineral nutrients, farming communities lost interest in the use of humic substances. One of the reasons was the fact that mineral nutrients used as a source of plant nutrition offered better yields than organic fertilizers. The decline in the use of organic fertilizers may also be due to what would be called the 'Great Disappointment' resulting from the exaggerated claims as to the miracle of their effectiveness. The marketing of low quality, highly priced products, worsened the situation. On the other hand there has been a scientific controversy surrounding the nature of humic substances and their functional roles, which somehow are difficult to understand as compared to the basic knowledge on fertilizers (New AG International, 2003).

Humic substances are known to be potential raw materials for production of humic acids. Currently, literature does not say much about the large-scale production of humic acids. Despite the many attempts to produce humic and fulvic acids at a large scale, very few

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countries have made a breakthrough. In South Africa the production of humic acids from black liquor is currently conducted by Lignotech an industry partner with Sappi. The large-scale production of high quality humic acids would require sustainable sources of raw material and a reliable method of extraction.

Extraction procedures are bound to alter some of the chemical linkages present in humic substances (mainly ester bonds in biopolyesters such as cutins and suberins) (Wikipedia, 2006). Jenkinson and Tinsley have shown that humic material from different sources have very different infrared spectra; and Makstnow and Liwski have demonstrated differences in plant response to humic fertilizers according to their method of preparation (Senn and Kingman, 1973). It is therefore to be expected that the source and method of extraction would be especially important in deciding the soil fertility potential of the humic acids.

### 1.3 OBJECTIVES OF THIS STUDY

The study was carried out with an aim to extract and analyse humic acids from raw materials suitable for large-scale production. The study focused mainly on four specific objectives:

Objective 1: Testing the hypothesis that black liquor is the potential source for the large-scale production of humic acids. The main focus of this objective was to compare the production yields and E4/E6 ratios of humic acids extracted from black liquor against other potential sources randomly identified.

Objective 2: assess four extraction methods for the extraction of humic acids (HA) from the preferred source, i.e. BL namely:

- Humic acid precipitation method (HAp): a simple precipitation method based on acid-base reactions.
- Lignin extraction/humic acid precipitation method (LHAp): a simple precipitation method based on acid-base reactions followed immediately after lignin has been removed from the extracted sample material.
- Humic acid precipitation/acid treatment method (HAp): the humic acid sludge produced by a humic acid precipitation method is treated three times with HCl to remove salts.
- Lignin extraction/humic acid precipitation/acid treatment method (LHAp): humic acid sludge produced by LHAp method is treated three times with HCl.

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All methods were formulated based on the standard precipitation method recommended by the International Humic Substances Standard (IHSS, 2005) for the production of humic acids. The idea was to identify the extraction method that will be most suitable for the large-scale production of humic acids.

Objective 3: investigate the effect of extracted humic acids on plant growth.

## **CHAPTER 2. LITERATURE REVIEW**

### **2.1 INTRODUCTION**

Humic acids are extracted from various sources which include humic substances. The ability to extract substantial amounts of humic acids from raw materials depends largely on the chemical composition of the raw material, and effectiveness and efficiency of the extraction methods. This chapter provides a review of some of the critical factors that should be considered for effective and efficient extraction of humic acids from various raw materials for large-scale production and application.

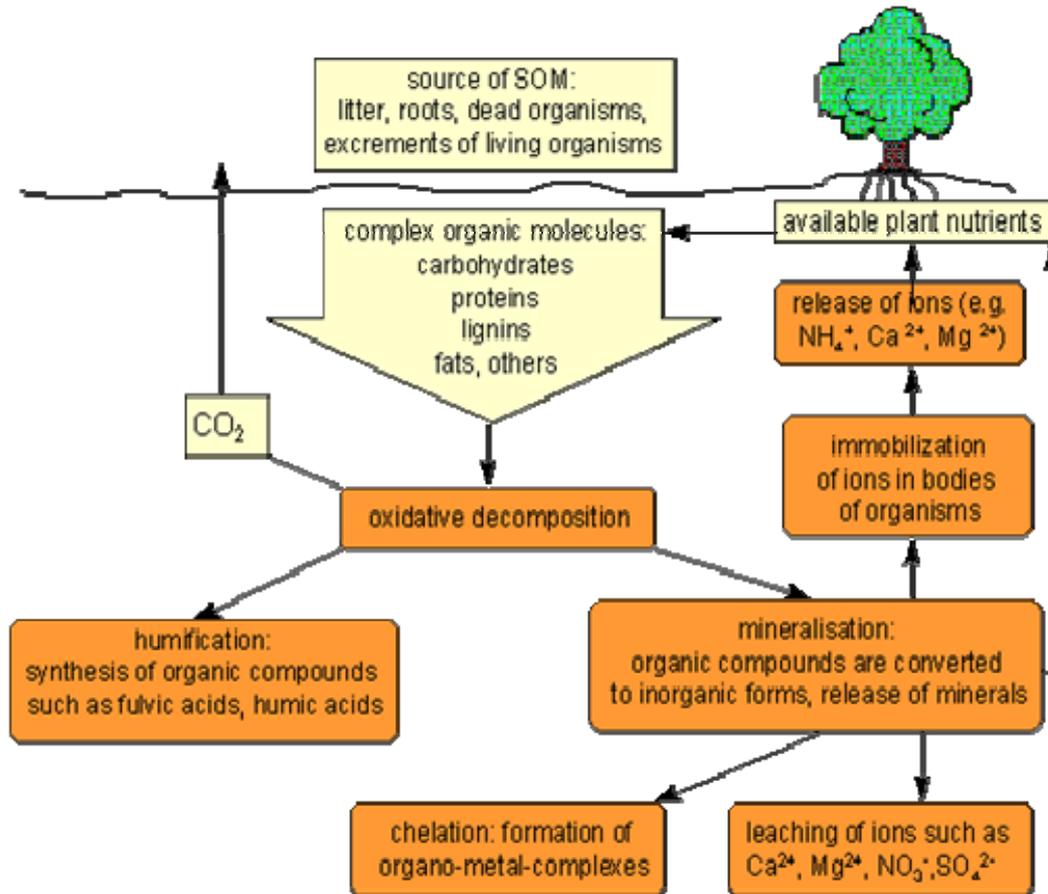
### **2.2. THE GENESIS OF HUMIC SUBSTANCES**

Humic substances are the dark-brown fully decomposed (humified) remains of plant and animal organic matter. They are the most chemically active compounds in the soil, with cation exchange capacities far exceeding those of clay. The genesis of humic substances is one of the least understood subjects of humus chemistry. In natural processes humic substances are formed through the process called humification (see Figure 2.1). Humification is a natural process of changing organic matter such as leaves into humic substances by geo-microbiological mechanisms (Mayhew, 2004). This process begins when organic residues from plants and animals come in contact with microbial life in the soil (U-Mate Int. Natural Organic Humates, 2004). During humification, microbes utilize carbon compounds for their own metabolism with the undigested portion of residue accumulating as humus. However, humus is not completely immune to decomposition. Eventually microbes recycle all elements in humus back to their origin, though it may take a long time to do so (Heng, 1991). Unlike most other natural biosynthetic processes, humification occurs in a complex, chaotic system where there is no closed control of the process by enzymes, cell structures, membranes or cellular transport systems.

Organic matter mostly contains complex organic molecules, carbohydrates, proteins, lignin, fats and others. It has been observed that there is a chemical relationship between lignin and humus (Fischer and Schiene, 1990).

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The degradation of the lignin components of plant material through such microbial degradation is critical to humic substance formation.

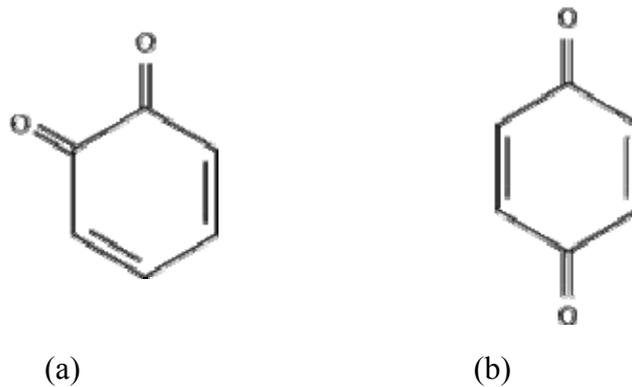


**Figure 2.1: Humification process of soil organic matter.**

The assimilation of nutrients from the organic residues by microorganisms constitutes the first stage of forming humus. To a large extent, considerable quantities of nutrients and energy assimilated into the bodies of microorganisms are reused by other microbes when they die. This process leads to the formation of complex chemical structures, which are more stable than the chemical structures of the starting material. When the decomposed organic matter reaches a certain level of humification, it can be referred to as humus (humic substances) which is a mixture of complex organic compounds (humic acid, fulvic acid and humins). Several pathways have been postulated to explain the genesis/formation of humic substances during the decay of plant and animal remains in soil. However, more recent theories attribute the generation of humic substances to the release of phenolic aldehydes and acid during microbial attack of lignin, cellulose, and other non-lignin substances. In this case, the phenolic compounds are converted into quinones before they polymerise to form humic-like

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macromolecules (Flaig et al., 1975; Stevenson, 1982). As a consequence, this has led to the application and utilization of lignin as plant nutrients. The popular classical theory argues that humic substances represent modified Lignins (Weber, 2005). The mechanisms involving quinones (Figures 2.2) are also favored.



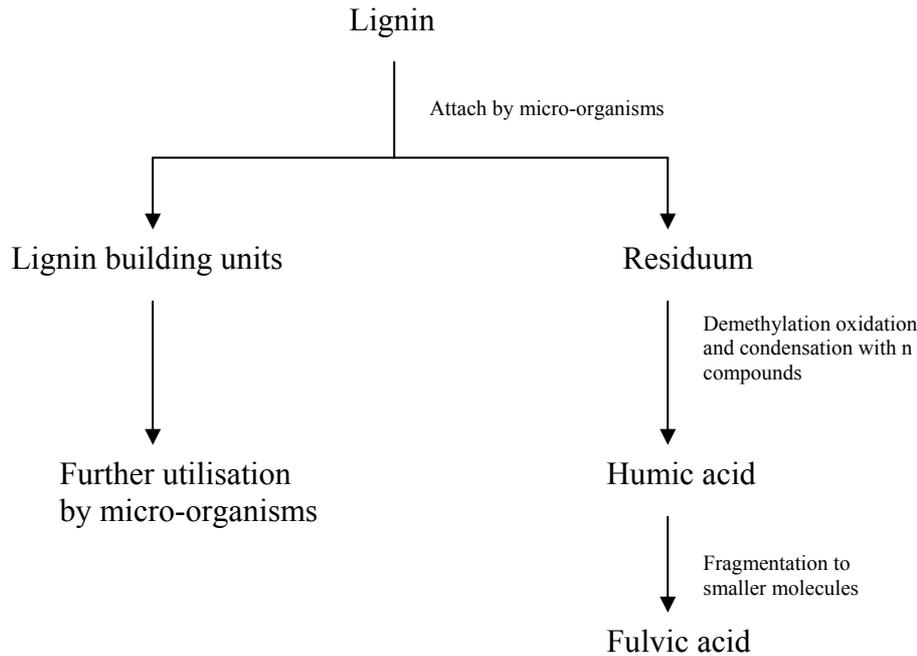
**Figure 2.2: The structure of quinone (a) orthobenzoquinone (b) parabenzoquinone.**

However, regardless of the degree at which the pathway is favored, all pathways considered are feasible for the synthesis of humic substances in nature. Nevertheless, their contribution may differ from one environment to another, for example; a lignin pathway may predominate in poorly drained soils and wet sediments, whereas synthesis from polyphenols may be of considerable importance in certain forest soils (Weber, 2005).

### 2.2.1 The lignin theory (Pathway 1):

This theory assumes that lignin is incompletely utilized by the microorganisms and the residue becomes part of the soil humus. The modification of lignin includes the loss of methoxy groups (-OCH<sub>3</sub>) with the generation of o-hydroxyphenols (C<sub>6</sub>H<sub>5</sub>OH) and carboxyl groups (-COOH). This modified material is what comprises humic and fulvic acids. Figure 2.3 is an illustration of the lignin theory pathway as presented by Waksman (1932).

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**Figure 2.3: The lignin theory of humic formation (Waksman 1932).**

The following evidence was cited by Waksman (1932) in support of the lignin theory of humic acid formation:

- Both lignin and humic acid are decomposed with considerable difficulty by the great majority of fungi and bacteria.
- Both lignin and humic acid are partly soluble in alcohol and pyridine.
- Both lignin and humic acid are soluble in alkali and precipitated by acids.
- Both lignin and humic acid contain  $-OCH_3$  groups.
- Both lignin and humic acid are acidic in nature.
- When Lignins are warmed with aqueous alkali, they are transformed into methoxyl-containing humic acids.
- Humic acids have properties similar to oxidized Lignins.

Although it is true that lignin is less easily attacked by microorganisms than other plant components, mechanisms exist in nature for its complete aerobic degradation (Weber, 2005).

### 2.2.2 The polyphenol theory (Pathways 2 and 3)

Pathways 2 and 3 are somewhat similar except that polyphenols in pathway 2 are synthesized by microorganisms from non-lignin carbon (C) sources like cellulose. Polyphenols are then

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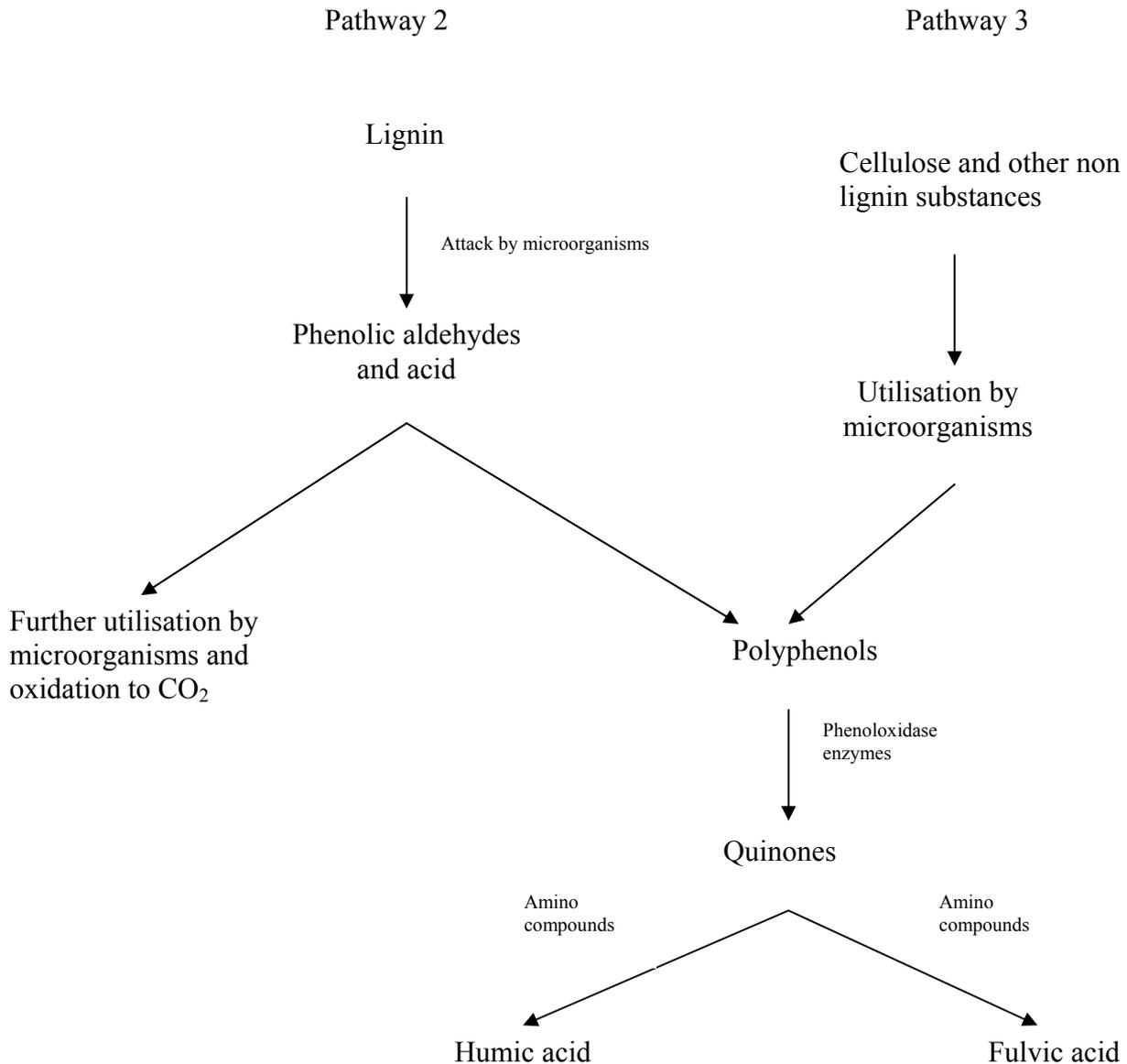
enzymatically oxidized to quinones and converted to humic substances. Quinones of lignin origin, together with those synthesized by microorganisms are assumed to be the major building blocks from which humic substances (humic and fulvic acids) are formed in pathway 3, cellulose and other non-lignin materials are the starting materials. In this pathway lignin still plays an important role in the humus synthesis, but in a different way from the lignin theory. Phenols, aldehydes, and acid released from lignin (starting material for pathway 2) during microbial attack undergo enzymatic conversion to quinones shown in Figure 2.2. These quinones in turn polymerize in the presence or absence of amino compounds to form humic-like micromolecules (Stevenson, 1982). Figure 2.4 shows lignin, cellulose and other non-lignin substances as possible sources of phenols for humus synthesis.

In Figure 2.4, lignin is freed of its linkage with cellulose during the decomposition of plant residues and subjected to oxidative splitting with the formation of primary structural units such as derivatives of phenyl propane. The side chains of lignin-building units are then oxidized and demethylation occurs. The resulting polyphenols are converted into quinones by polyphenoloxidase enzymes. These quinones arising from lignin and probably from other sources react with nitrogen-containing compounds to form dark-coloured polymer humic substances, which is a combination of humic and fulvic acids.

### **2.2.3 Sugar-amine condensation (Pathway 4):**

According to pathway 4, reducing sugars and amino acids, which are formed as by-products of microbial metabolism, undergo nonenzymatic polymerization to form brown nitrogenous polymers of the type produced during dehydration of certain food products at moderate temperatures. However, there is an objection to this theory because the reaction proceeds slowly under the temperatures found under normal soil conditions. On the other hand, drastic and frequent changes in the soil environment (freezing and thawing, wetting and drying) coupled with the intermixing of reactants and mineral materials having catalytic properties may facilitate condensation (Weber, 2005).

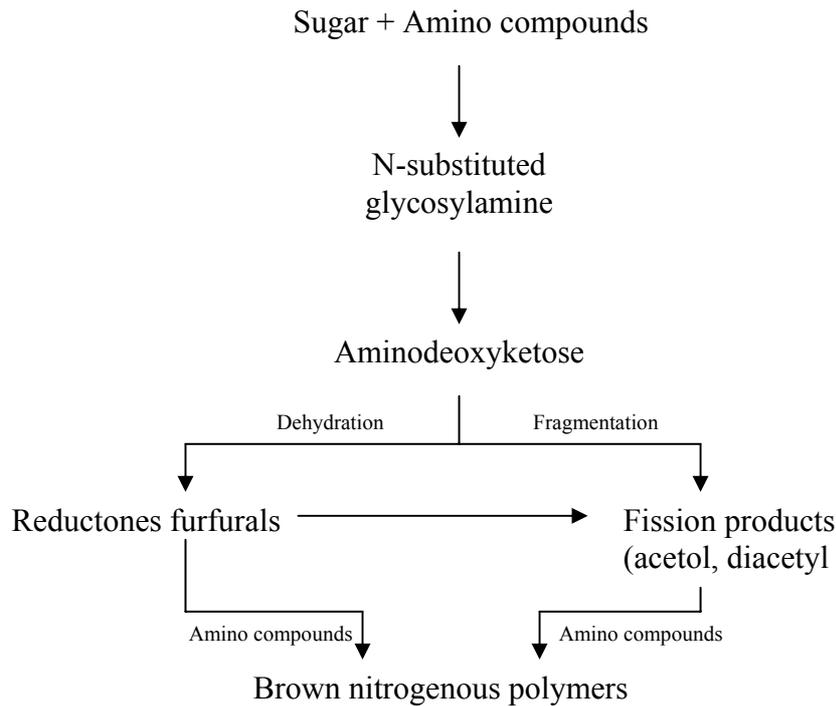
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**Figure 2.4: The polyphenol theory of humus formation (Stevenson, 1982).**

An attractive feature about this theory is that the reactants (sugars, amino acid, etc.) are produced in abundance through the activities of microorganisms. Figure 2.5 shows the sugar amine condensation pathway as outlined by Stevenson (1982). During the initial sugar amine condensation, amine is added to the aldehyde group of the sugar to form n-substituted glycosylamine. Glycosylamine is in turn oxidized to form n-substituted-1-amino-deoxy-2-ketose.

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**Figure 2.5: Sugar amine condensation (Stevenson, 1982).**

This is subject to fragmentation and dehydration processes. Fragmentation results in the formation of 3-carbon chain aldehydes and ketones such as acetol, diacetyl, etc., while dehydration leads to the formation of reductones and hydroxymethyl furfurals. These compounds readily polymerize in the presence of amino compounds to form brown-coloured products, particularly humic and fulvic acids (Stevenson, 1982).

#### 2.2.4 Wood Processing

In addition to the above mechanisms, industrial processes involving wood processing, such as the transformation of wood into usable fiber in pulp and paper industry (Duarte et al., 2003) is another way through which humic substances can be formed. These pulp processes always involve the degradation of plant chemical components such as cellulose, sugars, lignin, etc. One of the major waste products of this process is lignin-derived compounds (Kringstad and Lindstrom, 1984) that have spectroscopic characteristics resembling those of humic substances (Duarte et al., 2003). Lignin derived compounds also exhibit analytical difficulties in chemical characterization similar to those of humic substances (Virkki, 1992).

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**2.3 SOURCES OF HUMIC SUBSTANCES**

As depicted in Figure 2.1 humic substances may originate from litter, roots, dead organisms and excrements of living organisms found in either soil or water. Traditional researchers have been isolating humic substances from soils and water (Bortiatynski et al., 1996). These humic substances have been re-generated in the soil through practices such as crop rotation, planting legumes, plowing under green manure and application of compost. However, these practices are time consuming and costly, and today's economic pressures prevent growers from sustaining these practices. An analysis of this problem dictates that a rapid and economically practical solution is to identify a dependable supply of performance proven humic substances, and apply them directly to the soil and, in many cases, the plant itself (HumaTech, 2002). Besides soils and water, humic substances can be found in varying concentration in a number of different sources (Table 2.1) (Mayhew, 2004). Some of these sources are reviewed in this chapter.

Table 2.1: Different sources of humic substances with different concentrations (HuminTech, 2005).

<u>Natural Source</u>	<u>% Humic/Fulvic acid</u>
Leonardite	25 to 90
Compost	5 to 25
Peat	5 to 20
Peat Moss	5 to 20
Lignite	5 to 15
Manure	1 to 3
Soft coal	2 to 5
<u>Hard coal</u>	<u>0 to 1</u>

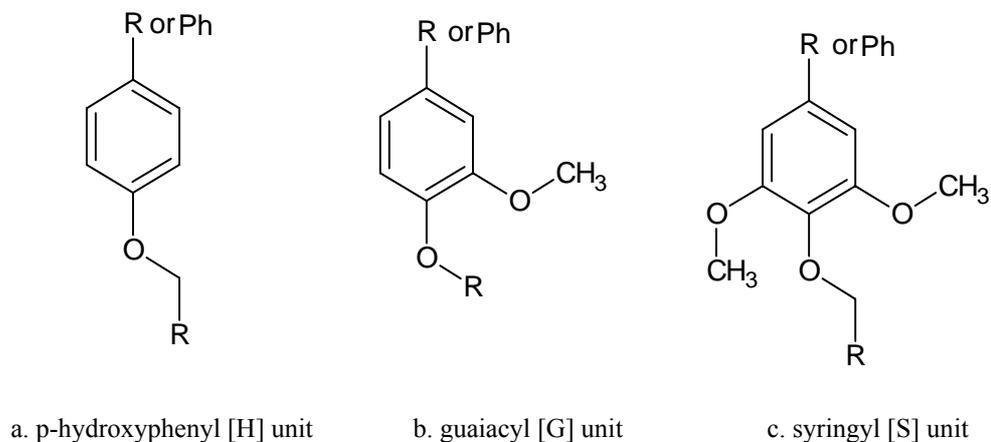
Table 2.1 presents the humic and fulvic acid contents of various natural sources expressed in percentage ranges. In this Table, it is shown that there is a wide distribution of humic substances on the face of the earth. However, Leonardite so far has shown to be the best source of humic substances ranked by humic and fulvic acids concentrations. In this study, other potential sources were investigated.

**2.3.1 Lignin**

Lignin, as indicated earlier, is a potential source of humic substances. Lignin is a polymer composed of substituted phenols with 3-C side chains (Sonnenberg et al., 1999) and is amongst the most abundant, renewable products on earth. It is a polyphenolic structure which functions as cement between xylem and phloem vessels and is mainly found in vascular plants.

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The chemical makeup of lignin varies between different plant families. In dicotyledonous angiosperms, lignin is composed of guaiacyl (G) and syringyl (S) monolignol units (see Figure 2.6) while in gymnosperms, they lack syringyl monolignol (Adler, 1977). However, regardless of wood material from which the lignin is derived, its structure shows a network of aromatic units and aliphatic molecules with various linkages. Figure 2.7 demonstrates various linkages that are found in various lignin molecules. The resistance of the lignin molecule against degradation depends on the type of available linkages on the molecule (see Appendix 8.2, Table 8.2.1).



**Figure 2.6: Various units of lignin molecule.**

The ether and  $\beta$ -O-4 linkages (see Figure 2.7) (Plant Physiology, 1999) are both frequent and labile, rendering them a target for the delignification process. In contrast, carbon-carbon linkages are resistant to microbial degradation especially the biphenyl 5-5 bonds involving the aromatic C-5 position, which is available for inter-unit linkages only in G units (Adler, 1977). Thus, conifer wood Lignins essentially made of G units are less susceptible to Kraft delignification than deciduous wood Lignins comprising both G and S units (Chiang et al., 1988). Fractions of plant tissues which are resistant to microbial degradation, such as lignified tissues, are altered superficially in the soil to form humic substances. Properties of the humic substance formed are influenced by the characteristics of the original plant material (Schnitzer and Khan, 1978), as well as the method of degradation used for its production, i.e. which would either be microbial degradation in natural environments, or chemical degradation such as in the Kraft pulp process.

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**2.3.2 Oxidised sub-bituminous coals**

Leonardite, the sedimentation layer of soft-brown coal, and similar materials from oxidised sub-bituminous coals and carbonaceous shales as shown in Table 2.1 are reported to be good sources of humic substance materials (HuminTech, 2005).

Leonardite contains high concentrations of humic acids and higher content of carboxyl groups than other materials (Table 2.1 and 2.2). It resembles an organic matter which has not reached a state of coal usually called soft brown coal, which is also another source of humic acids. It is the high oxidation degree of Leonardite that makes it unique from soft-brown coal (HuminTech, 2005). Compared to other organic products, Leonardite is very rich in humic acid. Leonardite also differs from other sources of humic acids because of its extremely bioactive nature (about 5 times stronger than other organic matter) owing to its molecular structure.

Table 2.2: Group content of Leonardite vs. brown coal.

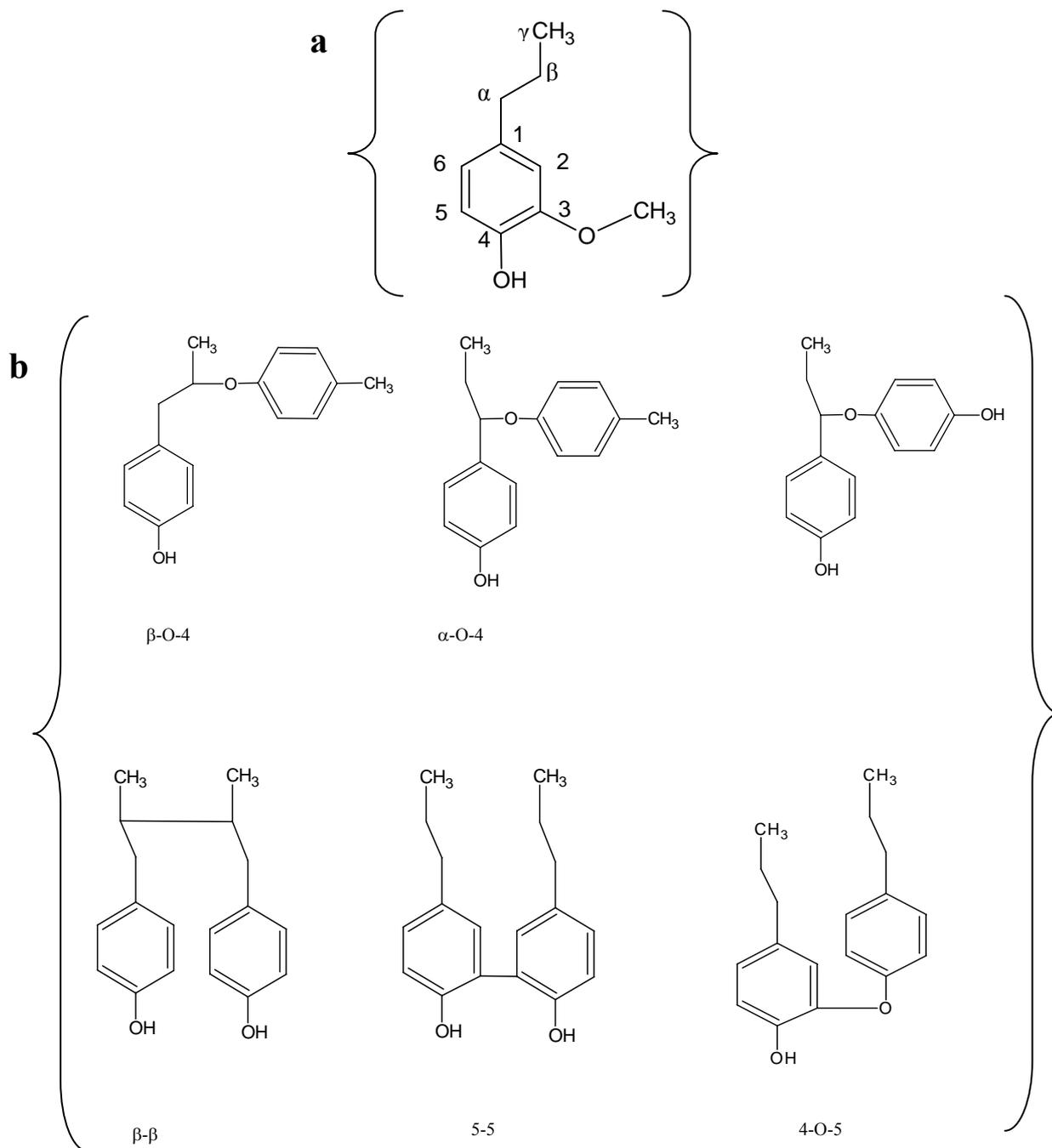
C	COOH	OH	CO	OCH <sub>3</sub>	Inactive groups	Total
Brown coal	73.8	9.2	4.8	4.3	0.5	19.9
Leonardite	63.9	17.4	4.8	4.2	0.5	28.4

Table 2.2 (HuminTech, 2005) shows that Leonardite has a high content of –COOH which makes it more active than other humic substances in the soil.

**2.3.3 Black liquor**

Over the past few years chemical pulping has increased on a global scale, and so has the amount of lignin produced as a by-product (Fischer and Schiene, 1990). Many efforts have been made in the pulp and paper industry in optimizing material utilization e.g. burning of technical lignin for fuel. During pulping process the presence of cooking chemicals such as hydrosulphide ions, hydroxyl ions or sulphate ions accelerate dissolution of lignin present in wood without increasing the dissolution of cellulose.

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**Figure 2.7: A view of lignin segments (a) polyphenyl propane (b) lignin molecule linkage units (Lignin, 2006).**

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**Figure 2.8: Brown coal (Leonardite) deposits.**

The small molecules produced from lignin during this process are no longer able to function as “cement” or fail to remain as part of the wood structure. They are instead dissolved in the cooking liquor to form the lignin fraction and humic substances (Mimms et al., 1993). Ideally, only lignin is supposed to dissolve during pulping process, but this is not the case. Both cellulose and hemicellulose react with hydroxyl ions during cooking and this reaction results in carbohydrate degradation into smaller, soluble molecules. These reactions are unwanted during the pulping process because they have a tendency to lower the pulp yield (Gratzl and Chen, 1999). However, the major components found in black liquor are lignin derived compounds (Kringstad and Lindstrom, 1984). When lignin in black liquor is efficiently burnt as a fuel, valuable water resistant adhesives are produced (Gratzl and Chen, 1999).

In circumstances where lignin is to be used as a source of humic substances and nutrients for plants, the following factors should be taken into consideration (summarized from Fischer and Schiene, 1990):

- Due to the chemical pulping process, technical lignin differs greatly from natural lignin. This is due to the differences in the number of C-C and C-O-C bonds, functional groups as well as the molecular weight and molecular weight distribution.
- Though technical lignin is the main component of spent liquors of chemical pulping processes, these liquors contain several other components rendering it a mixture of substances of a complicated and variable composition, particularly for organic and inorganic components. Organic components consist of low molecular weight

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substances from degradation reactions of the entire wood substance and special derivative from the polysaccharides and extractives of the wood species. The inorganic components comprise of pulping chemicals and their conversion products as well as the ash components of the wood species. If the spent liquors are directly applied to the soil, undesirable side effects may occur hence the need to isolate the humic substances.

- Technical Lignins and those in the form of spent liquor contain insufficient amounts of macronutrients for their use on crop production, especially nitrogen and phosphorus.

Sulphite spent liquor was reported by Melms and Schwenzon (1967) as the first liquor to be investigated for organic fertilizer. Then neutralized sulphite spent liquor was reported to have a positive effect on soils deficient of humus. Melms and Schwenzon (1967) also observed that, sulphite spent liquors contain very little nitrogen as well as phosphorus. This made the sulphite spent liquors unreliable sources of humus. In order for this liquor to be utilized as organic matter, compost of sewage water, sewage sludge and waste material (garbage) had to be added. An alternative approach was the mixing of spent liquor with conventional fertilizers.

Fischer and Scheine (1990) conducted several experiments aimed at producing organomineral fertilizers, i.e. products characterized by relatively high N content and a low C/N ratio. In their attempt, spent liquors from pulping process were used as raw materials. It was reported that both sulphite and alkali spent liquors were converted to fertilizer products with nitrogen content of up to 24% (Fischer and Scheine, 1990). In general black liquor may constitute a reliable source of humic substances from which humic acids could be extracted

### **2.4 STRUCTURE AND PROPERTIES OF HUMIC ACIDS**

Humic substances contain macromolecules with amino acid, amino sugars, peptides, and aliphatic compounds involved in linkages between the aromatic groups. Their nomenclature suggests that they are acids that form actual salts. When the cation exchange sites on the humic molecules are filled predominantly with hydrogen ions, the material is considered to be an acid and is named accordingly. However, it has no great impact on the pH because the acid is insoluble in water. When the predominant cation on the exchange site is not hydrogen, the material is called humate. The humates of monovalent alkali metals are soluble in water, but those of multivalent metals are insoluble. Because of their effect on the solubility of the

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materials and absorption by clays, different cations have little effect on the humic molecules (Schnitzer and Khan, 1978).

Figure 2.9 shows the results of electron microscope observation of humic acids (Drozd, 1978). Several workers such as Flaig and Beutelspacher (1975), and Visser (1964) have used electron microscope for observing shapes and sizes of humic acid particles (Schnitzer and Khan, 1978). In Figure 2.9 it is shown that humic acids consist of heterogeneous mixture of compounds, therefore, they have no single structural formula. The electron microscope shows a loose spongy structure with many internal spaces.

Generally, results from the electron microscope showed that an increasing pH of humic substances in solution causes the forces that bind the molecules together to weaken. This increases the ionization of -COOH and phenolic -OH groups causing the particles to separate and begin to repel from each other electrically (Schnitzer and Khan, 1978). This condition has potential to improve soil aeration and complex formation on application of humic substances as soil conditioners.

The hypothetical structure for humic acid shown in Figure 2.10 contains free and bound phenolic -OH groups, quinone structures, nitrogen and oxygen as bridge units and -COOH groups variously placed on aromatic rings. From Figures 2.10 it is evident that humic acids are mainly composed of carboxylic and hydroxyl groups as their functional groups.

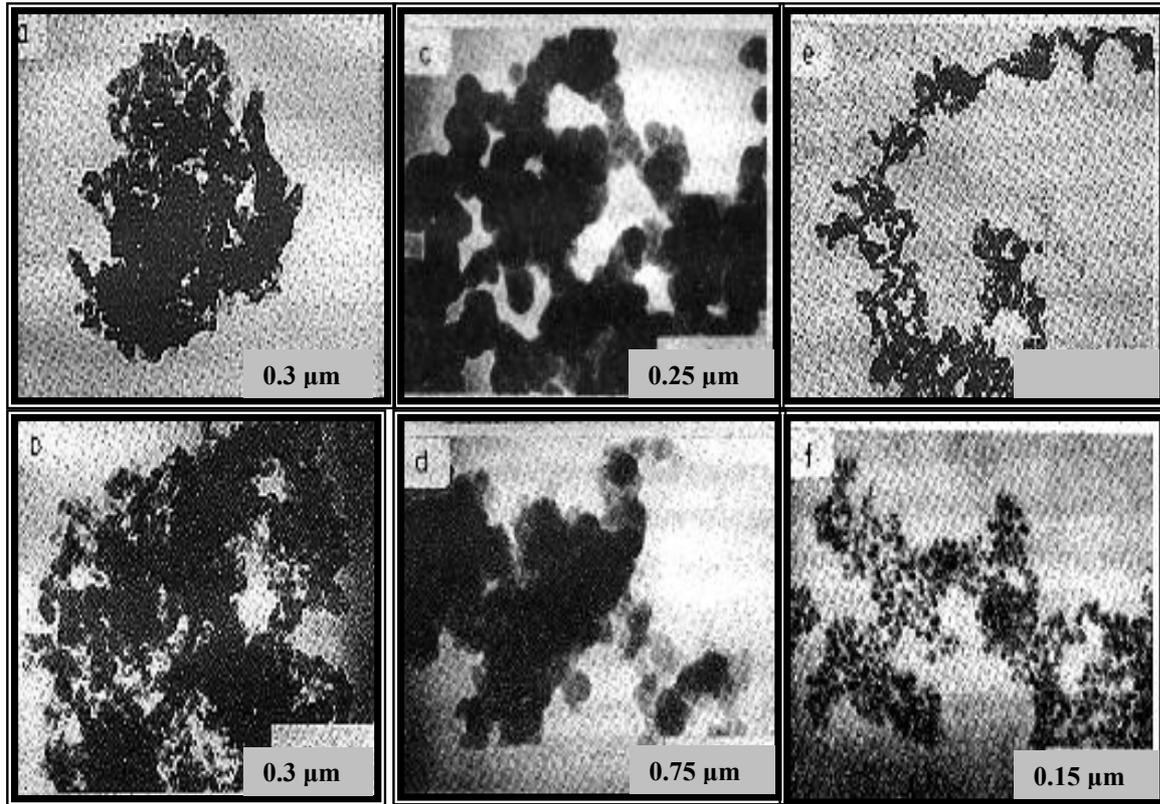
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Humic acids of peaty sandy hydro earth soil

Humic acids of haplic phaeozems

Humic acids of podzolic soil

Figure



2.9: Electron microscope observations of humic acids (Drozd, 1978).

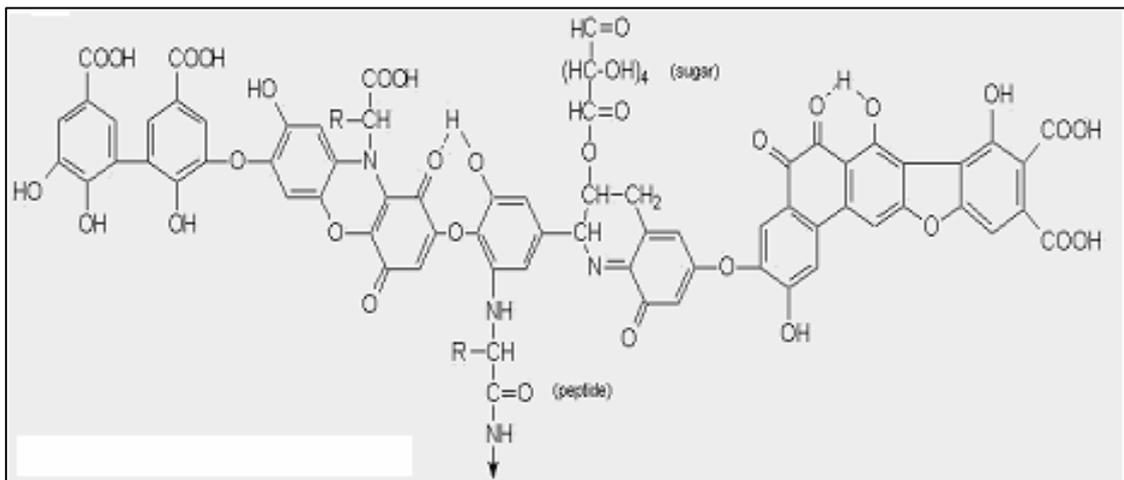


Figure 2.10: Hypothetical model structure of humic acid (Stevenson, 1982).

Some laboratory findings showed that up to 50% of the aliphatic structures in humic acids consist of n-fatty acid esterified to phenolic -OH groups (Ogner and Schnitzer, 1970). The

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remaining aliphatics are made up of more “loosely” held fatty acid and alkane structures. These structures appear to be physically adsorbed onto the humic materials which structurally are not humic components but possibly aliphatic chains joining aromatic rings (Schnitzer and Khan, 1978).

The specific functional groups of humic acids are responsible for chelating various compounds in the environment, thereby improving nutrient utilization and preventing metal toxicity in waters, soils, and possibly in plants, animals and humans as well. Humic acids from peats show significant levels of phenolic carbons (C6), and methoxy carbons (-OCH<sub>3</sub>), associated with the presence of lignin-like material (Frimmel and Christman., 1988) when lignin is the starting material of humic acid. Part of humic acids phenolic compounds however, act as respiratory catalysts (Senn and Kingman, 1973). This variable molecular composition of humic acids allows a wide range of dissociation constants for the metals that are chelated by humic acids (Humet Products Documentation and Technical Information, 1999). Different metals are bound to humic acid with varying strength, which means that a particular metal chelate-bond modifies the binding stability of the other metal linkages. Due to the heterogeneous molecular composition of humic acids, a given metal may bind very strongly, while another may affix or release the same metal much easier (Humet Product Documentation and Technical Information, 1999). This particular metal binding capacity of humic acids is exemplified by the fact that when some alkali metals, such as K and Na, are bound by previously empty functional groups, the chelate bonds of Fe and Al may rupture easier than if the molecule contains an alkali earth metal, such as Ca (Humet Product Documentation and Technical Information, 1999). This is why vegetation suffers from microelements deficiency in the presence of Ca-humate in the soils although the needed elements are abounding in the humus.

This particular metal binding capacity also protects plants owing to the ability of water soluble fractions of humic substances (humic acid) to form precipitates with a number of metals (Ca, Cd, Hg, Pb, Ba), which leads to the formation of insoluble complexes. The complexes formed are not available to the plants and the concentration of toxicants in the soil solution is reduced (Frimmel and Christman, 1988). The pH, ionic strength, molecular weight and functional groups content are all factors influencing the quantity of metal ions bound by humic substances (Aiken, 1985).

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**2.5 POTENTIAL SOURCE OF HUMIC SUBSTANCES IN SOUTH AFRICA**

South Africa's pulp industry has grown substantially especially since the Second World War. It is now an integral part of country's economic structure. Since 1946 the annual consumption of paper and board in South Africa has risen (Association of Pulp, Paper and Board Manufactures of South Africa, 1977). An increase in the production process unfortunately results in large production of black liquor material, which may lead to disposals problems.

With such large amounts of black liquor in South Africa, the sustainable production of humic acids from black liquor is a possibility. The availability of great quantities of humic substances in black liquor makes black liquor a readily available raw material for the production of humic acids. Technically degraded lignin found in black liquor may be used for many purposes of which one is the production of humic agricultural products. This will provide a new and inexpensive source of humic substances in South Africa. The supply of black liquor from pulp industry measures in the millions of tons annually. In modern pulp industrial methods discussed in the following sections chemical pulping black liquor containing technical lignin is used as a valuable fuel and is also incorporated into the pulping chemical recovery system. Production of humic substances from black liquor is an effort hoped to provide a more effective utilization of this material in terms of remuneration.

**2.6 DESCRIPTION OF PULP AND PAPER INDUSTRIAL PROCESSES.**

The pulp and paper industry consists of complex technological processes that facilitate the transformation of wood into usable fibre (Duarte et al., 2003). During this process, pulp and paper are manufactured from raw materials containing cellulose fibers, generally wood, recycled paper, and agricultural residues. In most developing countries about 60% of cellulose fibers originate from non-wood materials such as bagasse (sugar cane fibers), cereals straw, etc. (World Bank Group, 1998).

Figure 2.11 shows a generic overview of the pulping process. The main process in paper manufacturing includes preparatory steps such as wood debarking followed by its chipping. The wood chips are then cooked in a biodigester to produce pulp, which is later bleached and made available for paper manufacturing. Cellulosic pulp is manufactured using mechanical and chemical means (Johnston et al., 1996). Generally, chemical processing is divided into two processes; Kraft or sulphate process (alkaline) and sulphite process (acid process). In chemical pulping process, chemicals are added into a pressure cooker containing wood chips

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and heat is applied. At this stage, lignin is dissolved to make it soluble in the cooking liquor. The pulp is washed to separate it from black liquor (also referred to as spent liquor) and wood waste (Blum, 1996). At this stage the pulp, called brown stock is ready to be bleached to completely remove lignin.

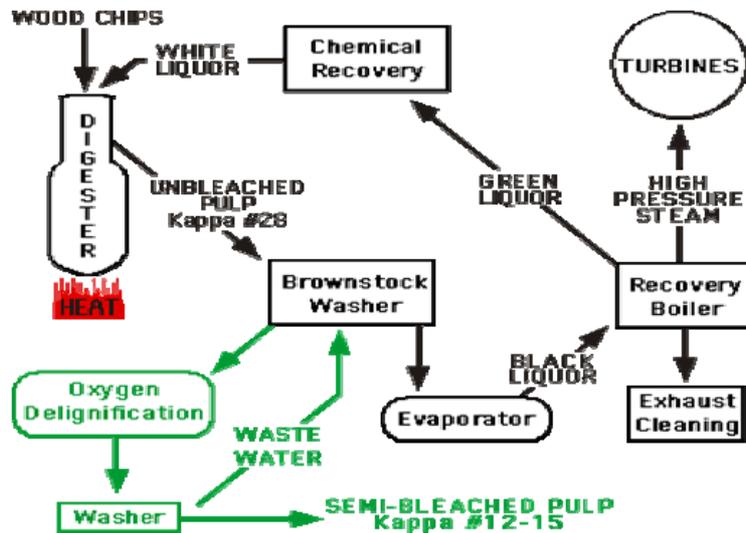


Figure 2.11: A general overview of the pulping process (Blum, 1996).

### 2.6.1 Sulphite chemical pulping (acid process)

The sulphite chemical pulp processes are outlined by Fengel and Wegener (1989). Sulphite processes consist of acid process, bisulphate process, multistage sulphite process, neutral sulphite process, and alkaline sulphite process. These processes are defined in terms of the composition of the cooking liquor, which in turn defines the process pH and the choice of cooking chemicals (acid or alkaline) used. In South Africa, Sappi Saiccor, situated in uMkhomazi, South of Durban is the industry leader in sulphite process (Moodely et al., 2003). Sappi Saiccor dissolves pulp using acid sulphite pulping process with calcium and magnesium bases (Thubron, 2004). Figure 2.12 outlines the Sappi Saiccor industrial process. The wood feedstock is milled and passed through the sulphite process to produce a high grade pulp. Four main streams of effluent are generated namely the calcium black liquor, the magnesium condensate black liquor) and two bleaching stage effluent streams.

The Mg-based black liquor condensate is burnt, both to recover the magnesium oxide present in this stream, and to generate energy, which involves evaporation of the effluent into thin liquor. This process leaves a condensate that forms during the evaporation process before the

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burning stage (Moodley et al., 2003). The Ca-based black liquor is passed on to Lignotech SA for lignosulphonates recovery, further processing and the production of lignin-based products (Thubron, 2004). Lignotech SA is a company that entered into a 50/50 joint venture with Sappi Saiccor to produce lignin by-products in South Africa in response to Sappi's environmental problems. Lignotech also produces a fulvic acid-based organic fertilizer from the Ca-based black liquor. The remaining effluent wastes from the process, mostly obtained from the bleaching train, are generally disposed into the sea. This resulted in severe environmental problems, which forced the company to find ways of reducing the quantities of generated effluents.

### **2.6.2 Kraft process**

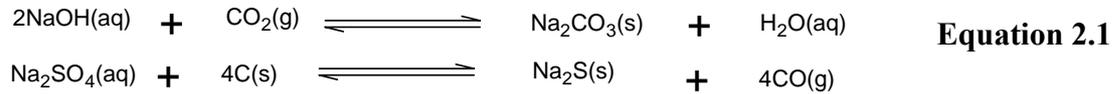
A schematic Kraft process design for Mondi Business Paper in Richards Bay is illustrated in Figures 2.14 and 2.15. Mondi Business Paper is a Kraft process that uses two major processes. These are alkaline (sulphate) process and soda process. In both processes, wood chips are cooked with sodium hydroxide in order to dissolve the lignin (Johnston et al., 1996). Sodium sulphate is an additional component of the pulping chemical mix in the Kraft process. The most important part of alkaline pulping process is the regeneration of cooking liquors (Fengel and Wegner, 1989; Minor, 1982). The recovery cycle in Kraft process is designed to recover pulp chemicals, reduce water pollution by combusting organic matter in the spent liquor, generate process heat and recover by-products.

The black liquor (used cooking liquor) is drained from the digester and evaporated into concentrated liquor which is combusted to produce mineral smelt. The mineral smelt is causticised to produce lime which is recycled back to the process. During the cooking process, half of the wood is dissolved (Gullichsen, 1991) which implies that there is high organic content in the black liquor hence black liquor becomes a suitable source of humic substances.



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In the recovery boiler, the organic content is combusted to produce heat. Carbon dioxide (CO<sub>2</sub>) reacts with sodium hydroxide (NaOH) to produce sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) which is an inorganic carbon. The added sulphate is reduced to sodium sulphite (Na<sub>2</sub>S) and hence the smelt produced by the boiler contains mainly sodium carbonate and sodium sulphite (Equation 2.1).



Sodium carbonate is dissolved in a tank to produce liquor which is subsequently filtered and treated with calcium hydroxide (slake lime) to convert sodium carbonate into sodium hydroxide. The resulting liquor is returned to the digestion process. The lime is regenerated through heating and mixing of these chemical with water recovered from the green liquor. This process is theoretically closed or environmentally friendly in relation to water use not with respect to atmospheric emissions, spills and condensate generation. The Kraft process is illustrated in Figures 2.13 and in 2.14.

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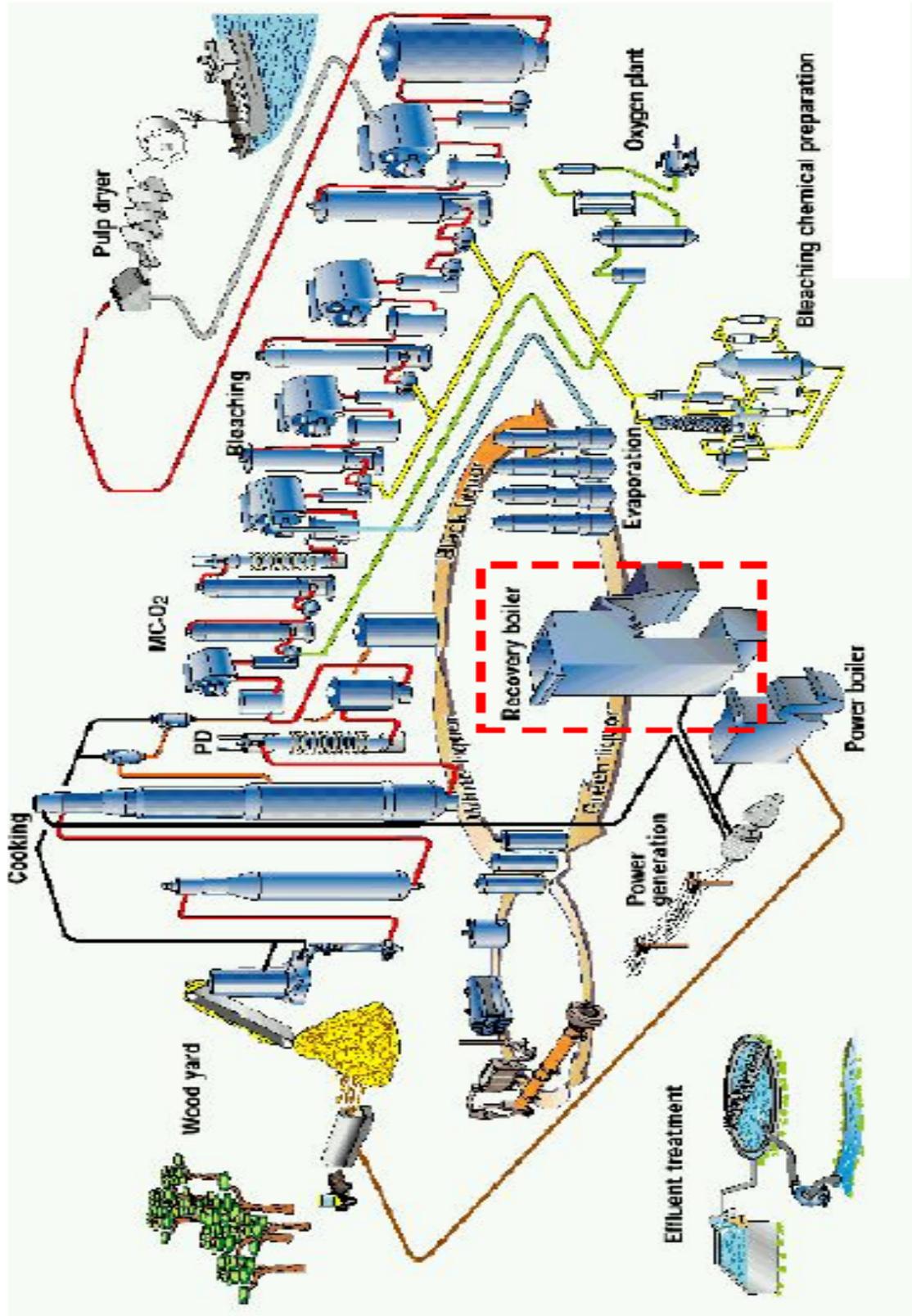
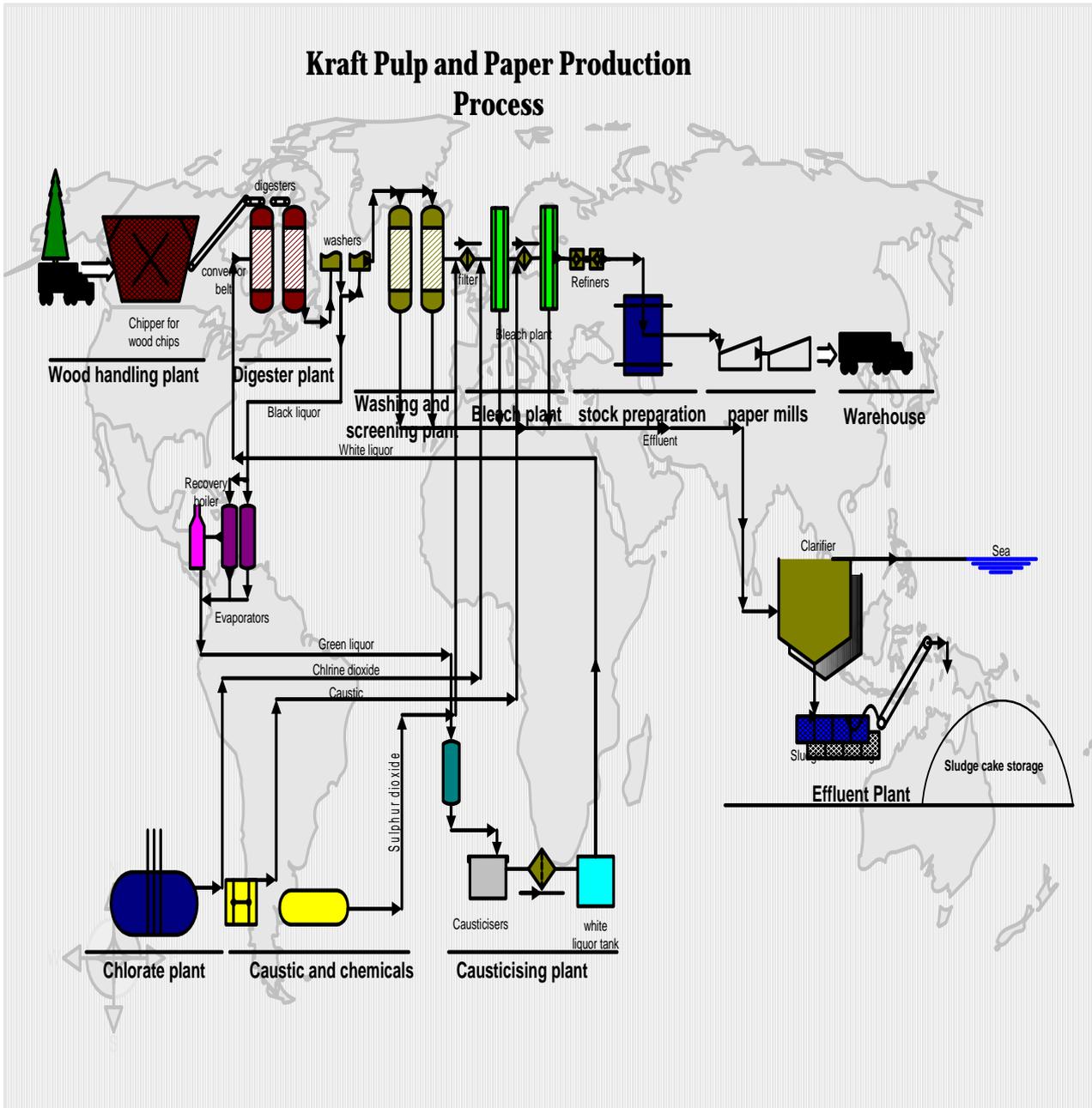


Figure 2.13: Schematic process flow diagram of Mondi (Gullichessen, 1991)

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**Figure 2.14: Schematic process flow diagram of Mondi Business Paper.**

## 2.7 ISOLATION/EXTRACTION OF HUMIC SUBSTANCES (HUMIC ACIDS)

A variety of methods for the isolation of humic acids from humic substances can be employed depending upon the nature of the material of humic substances. These methods include:

- The alkali extraction method which uses 0.1 M NaOH. This method is commonly used for the isolation of humic acids present in organic matter like composts and soils.

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- A method using mild extractants where several milder and more selective extractants like  $\text{Na}_4\text{P}_2\text{O}_7$  and EDTA are recommended alternatives for extraction with strong alkali.
- A method using formic acid ( $\text{HCOOH}$ ) where extraction of soil organic matter shows that under certain circumstances extraction of humic acids present in soil and compost humic substances respectively, can be achieved, etc. (see Table 2.3).

In this study, a basic precipitation process accompanied by the alkali-based process has been selected as the preferred method for the extraction of humic acids. This is due to its popular use as the standard method for the extraction of these humic substances. In Table 2.3 it is shown that NaOH is so far the best solvent of extraction available as it shows an efficiency of up to 80% extraction of humic substances.

Table 2.3: Reagents used for the extraction of humic substances (Stevenson, 1982).

Extractant	% humic substances extracted
NaOH	80
$\text{Na}_4\text{P}_2\text{O}_7$	30
Organic chelates: acetyloacetone, Cupferron, hydroxyquinoline	30
Formic acid ( $\text{HCOOH}$ )	55

Alkaline extraction process begins with humic bearing material which is treated with an alkali base (0.1 M or 0.5 M dilute NaOH) before an acid is added. The acid causes a coagulated black sludge-like material to precipitate out of the solution. The precipitate is called humic acid which can also be called a salt because it is a product of adding an acid to an alkaline solution hence the word humate is said to be more appropriate (Schnitzer, 1999). Although the use of alkaline solution has been criticized as it causes damage or medication of the chemical structure of humic substances under an atmosphere of nitrogen, no proof has been found to substantiate this claim (Schnitzer and Khan 1978). Schnitzer and Skinner (1968) extracted FA from a Spodosol Bh (a type of soil sampled from Pomona horizon in Japan) horizon under nitrogen with 0.5 N NaOH and with 0.1 M HCl (Kumada, 1987). Following the purification process, each extract was characterized using chemical and spectroscopic methods. The results showed that elemental composition of the two materials was very similar and the oxygen-containing functional groups had the same order of magnitude. It was also observed that the yield of humic material extracted and ash content appeared to be affected by the concentration of NaOH. A solution of 0.1 M NaOH was found to be more efficient than higher NaOH concentration (Ponomarova and Plotnikova, 1968; Levesque and Schnitzer,

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1967). However, in extracting humic materials low in ash content, they found that the most suitable solution must have a concentration of 0.4 M or 0.5 M NaOH. Neutral salts of mineral and organic acid such as 0.1 M Na-pyrophosphate solutions have also been used for the extraction of humic substances but the yields were found to be very low (Schnitzer and Khan, 1978). Schnitzer and Khan (1978) also showed that pyrophosphate was difficult to remove from humic materials during purification. Other attempts involving treating humic substances with chelating resins were employed. Levesque and Schnitzer (1967) established that humic materials extracted with chelating resins were more polymerized than those extracted with dilute alkali.

Duarte et al. (2003) extracted humic and fulvic acids fractions from Kraft pulp mill effluent using the XAD (Amberlite™ XAD™ resin) procedure. The Kraft pulp mill effluent was filtered through 0.45 µm membrane filters, acidified to pH 2.0, and then pumped through a column of XAD-8 resin. The adsorbed organic acids were back eluted with 0.1 M NaOH following the alkali extraction method and the eluate formed was immediately acidified ranging from pH 3.0-4.0. Humic acids were precipitated by acidifying the eluate to a pH of 1.0 and then separating them using centrifugation technique at more than  $1 \times 10^7$  g. Humic acids were re-dissolved in 0.1 M NaOH, under nitrogen atmosphere, and were immediately acidified to a pH 3.0-4.0. The humic and fulvic acids were then desalted to remove components such as NaCl. The solutions were freeze-dried and kept in desiccators over silica gel.

In their findings Duarte et al. (2003) discovered that fractions of higher molecular size exhibit a higher degree of conjugation of pi bonds. Humic acid fractions exhibited higher degree of conjugation of pi bonds especially in the highest size fractions. It was also observed that chemical composition of the fulvic acid fractions differed clearly from the humic acid fractions despite similar apparent molecular size.

In their experiment Neto et al. (2004) isolated polysaccharides dissolved in *Eucalyptus* globulus Kraft liquor using a precipitation procedure. Polysaccharides in black liquor were precipitated using glacial acetic acid. After two days the solution and the precipitate were separated by centrifugation and the solution decanted off. The precipitate was sequentially washed with 1,4-dioxane-water (2:1) solution, 1,4-dioxane, methanol and acetone and finally dried under vacuum with phosphorus pentoxide. As part of their findings, Neto et al. (2004)

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discovered that saccharides may be selectively precipitated as oligo or polysaccharides by black liquor acidification.

The procedure used for the extraction of humic substances can also extract considerable amounts of mineral components such as salts, sesquioxides and clays. Also, the organic constituents such as proteins and carbohydrates can be co-extracted as they are linked to the humic substances by covalent bonding (Stevenson, 1982). In a study that was conducted by Sanchez-Monedero et al. (2002) on the effects of HCl-HF purification treatment on chemical composition and structure of humic acids, they found that this purification method successfully reduced the ash content of humic acids. However, the differences between the average C, N, H and O (i.e. carbon, nitrogen, hydrogen and oxygen) concentrations of the unpurified and purified humic acids were no longer more than 10%.

### **2.8 FINGER-PRINTING AND ANALYSIS OF HUMIC ACID**

Several advanced analytical techniques have been applied to elucidate the structural features of humic substances (Duarte et al., 2003). Spectroscopic methods are widely applied in the study of HAs (humic acids) and have contributed considerably in the understanding of their chemical structure and properties (Sparks, 1999). They comprise of techniques such as ultraviolet visible spectroscopy (UV-VIS spec.), atomic absorption spectroscopy (AA) and Infrared (IR) spectroscopy for studying molecular compositions and vibrations. Infrared spectroscopic techniques generally do not provide direct identification of functional groups and structural entities of humic substances (HS). However, both UV-VIS and IR spectroscopic techniques do provide information that may be valuable in elucidating some structural and functional aspects of the chemistry of humic substances. Chromatographic analysis allows the separation of humic fractions available in the sample of a humic substance while elemental analysis will assist in elucidating their elemental composition such as C, H, S, O, and N content. A detailed description of these various analytical methods is explained below.

#### **2.8.1 Ultraviolet visible (UV-VIS) spectroscopy**

Ultraviolet visible spectroscopy is a well known and widely used tool for the identification of humic substances. This is frequently used for the qualitative and quantitative determination as well as characterization of changes in humic acid structures (Brauns and Brauns, 1960; Goldschmid, 1971). Absorption of humic acid in the ultraviolet range is based on the aromatic

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groups that contribute to the molecular structure, i.e. the sum of phenyl propane units, and on several chromophoric structural elements. In the UV region, absorbance is due to the contribution of  $\pi$ - $\pi^*$  electron transitions in the phenolic arenas, aniline derivatives, polyenes and phenolic aromatic hydrocarbons with two or more rings (Duarte et al., 2003). Although some differences exist in the UV-VIS spectra of different humic acids, the shape of the absorption curves emphasizes their characteristic nature, that is, whether they are aliphatic or aromatic. Structural differences in the spectral behavior of humic substances are revealed by the fine resolution of UV-VIS spectra (Borkman and Person, 1957; Sarkanen et al., 1967a,b,c; Norrstrom, 1972; Norrstrom, and Teder, 1971 and 1973; Lin and Detroit, 1981). The loss of aromatic structures of humic acid results in significant changes in the UV-VIS spectrum of these substances. Information about this behavior is observed through the extinction value which is a measure of electromagnetic radiation absorbed at a particular wavelength and peak maxima which is the highest point at the peak of absorption. A higher extinction value indicates increased transparency of the solution in which the humic substances is dissolved. This helps monitor the purity of a humic substance and to tell whether it has moved from the lignin state to a more humic state by finger-printing with a lignin molecule preferably isolated from the same material from which the humic substance was isolated. An increase in the degradation of aromatic structures causes a decrease in extinction values, the maxima flattens out, and finally the maxima at 280 nm disappears (Rydholm, 1965; Wegener, 1975). The predicative ability of UV-VIS spectroscopy data for humic substances and lignin characterization is mostly limited to the comparison of different Lignins and humic substance, evaluation of some special functional groups, and the determination of more severe structural changes due to chemical treatments.

The UV-VIS spectrum of humic acid is characterized by measuring the E4/E6 ratios in the ultraviolet visible spectrum of humic acid (Chen et al., 1977). The E4 is the absorbance measured at 465nm and E6 is the absorbance measured at 665nm of the UV-VIS spectrum. This technique is frequently used to determine the degree of condensation of aromatic network of humic acid structures (Kononova 1966). A low E4/E6 ratio indicates a high degree of condensation while a high E4/E6 ratio signifies a low degree of condensation assuming the presence of relatively large proportions of aliphatic structures. E4/E6 ratio is independent of the concentration of humic and fulvic acid in the sample, but does vary for humic substances extracted from different sources (Kononova 1966; Schnitzer and Khan, 1978). Salt concentrations e.g. NaCl can also have an effect on the E4/E6 ratio. The E4/E6 ratio decreases

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with an increase in NaCl concentration. UV-VIS spectra of humic acid are generally featureless as they have no well defined maxima or minima and their absorptivity tends to increase as their wavelength decreases as shown in Figure 2.15 (Kononova 1966; Schnitzer and Khan, 1978). However, a slight maximum is occasionally present in the region between 260 nm and 300 nm of the UV spectrum.

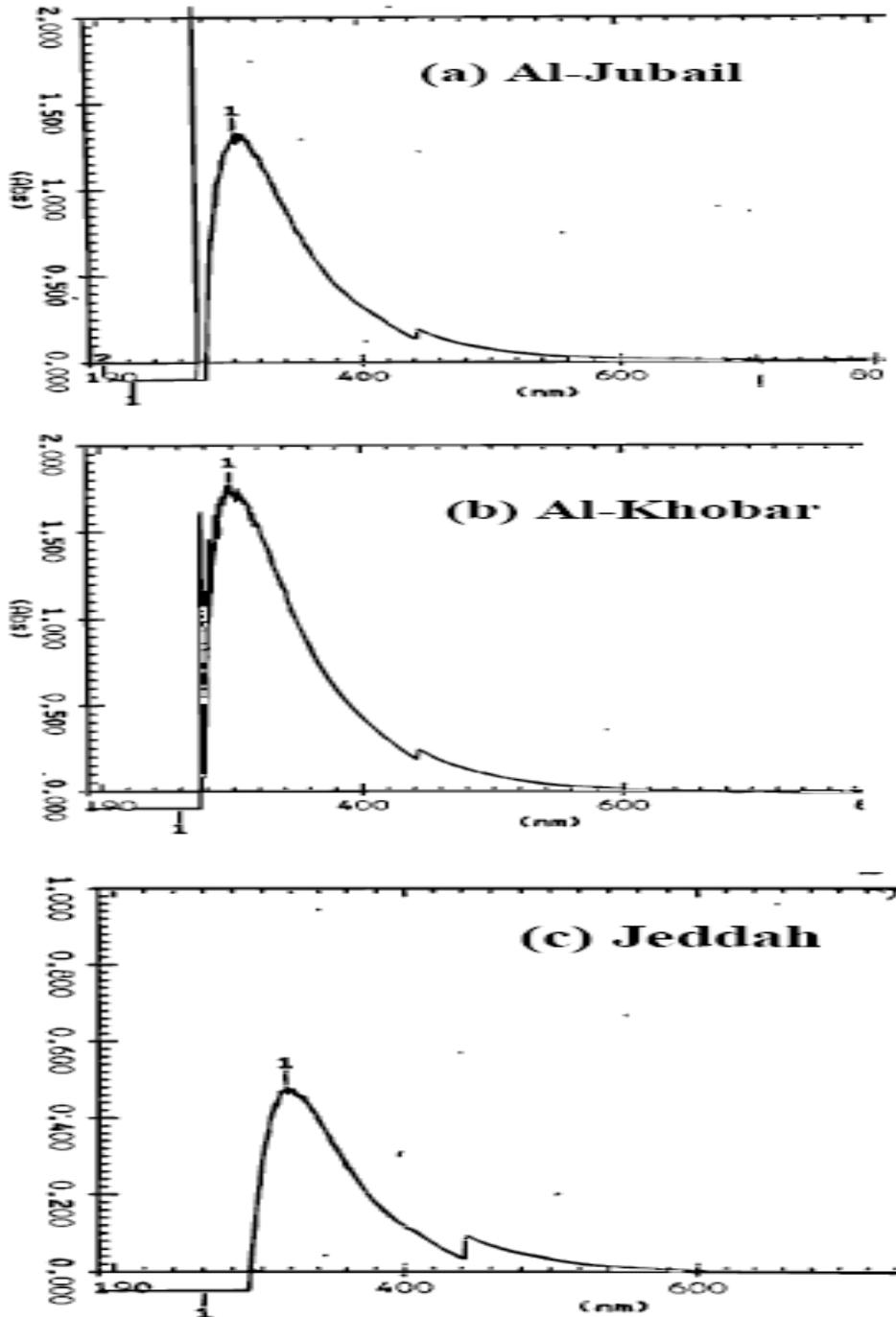
The UV-VIS spectra shown in Figure 2.15 were obtained using isolated humic substance from three sites: Al-Jubail, Al-Khobar and Jeddeah (Dalvi et al., 2000). A broad band having absorption peaks around 300 nm was observed irrespective of where the sample was collected. This absorption band can be used for the estimation of humic substances. In the study of humic acids from *Eucalyptus* bleached Kraft pulp mill effluent, Duarte et al. (2003) noted that the spectra of humic acids were very similar to those of other humic substances, decreasing monotonically with increasing wavelength.

However, in all spectra a shoulder in the region 250-300 nm (~280 nm) was observed and this was more prominent in the spectra of the low molecular size fractions. Using E250/E365 ratio similar to E4/E6 ratio, Duarte et al. (2003) observed that the unfractionated fulvic acid sample exhibited a higher E250/E365 ratio than that of the fractionated sample. In their study they discovered that there is a clear difference in the chemical composition of the fulvic acid fractions from the humic acids fractions though both substances had apparently similar molecular size.

### 2.8.2 Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectroscopy is another method for characterizing lignin and its derivatives such as humic substances. The infrared spectrum is a general method for finger-printing compounds with exactly known structures, although for humic substances several uncertainties are experienced with the interpretation of spectra (Zbinden, 1964; Hergert, 1971). This is because there are several variations in the structure of humic substances and chemical composition depending on the origin of the samples. This can also be attributed to variation in the technique of measuring humic substances either in suitable solvents, in the form of films, or in the form of potassium bromide pellets (Zbinden, 1964; Hergert, 1971). Table 2.4 shows the most probable IR bands of humic substances. The IR spectra of different humic substances are provided in Figure 2.16.

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**Figure 2.15: UV-visible spectra of humic substances from (a) Al-Jubail (b) Al-Khobar (c) Jeddah (Dalvi et al., 2000).**

The IR spectra of humic substances depict several major absorption bands which can be assigned to various structural groups (Ekman and Lindenberg, 1960; Wada, 1961; Metchell,

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1966; Higuchi and Kawamura, 1966; Sarkanen et al., 1967a; Sarkanen et al., 1967b; Sarkanen et al., 1967c; Hergert, 1971; Faix and Schweers, 1974). From Figure 2.16 it can be observed that humic substances show prominent bands in the regions of  $3150\text{-}3500\text{cm}^{-1}$ ,  $2800\text{-}3050\text{cm}^{-1}$ ,  $1550\text{-}1725\text{cm}^{-1}$ ,  $1300\text{-}1450\text{cm}^{-1}$ , and  $1000\text{-}1250\text{cm}^{-1}$ . The appearance of these peaks reveals the presence of various functional groups like carboxyl, phenolic, hydroxyl group derivatives of benzene and sulphur.

Duarte et al. (2003) conducted FTIR characterization of humic and fulvic acid fractions isolated from *Eucalyptus* bleached Kraft pulp mill effluent. In this analysis it was found that all fractions exhibited bands typical of lignin structural fractions (Duarte et al., 2003). Furthermore, the relative intensity of bands at  $\sim 1518$  and  $\sim 1114\text{ cm}^{-1}$  increased for fractions with low molecular sizes and these bands appeared to be more intense in the spectra of Lignins from *Eucalyptus* wood. These findings were attributed to the higher content of lignin-derived aromatic structures in fractions with low molecular sizes, compared to fractions with high molecular sizes.

Table 2.4: Important IR absorption bands of humic substances of lignin origin (Hergert, 1971).

Position $\text{cm}^{-1}$	Band origin
3 450-3 400	OH stretching
2 940-2 820	OH stretching in methyl and methylene group
1 715-1 710	C=O stretching nonconjugated to the aromatic ring
1 675-1 660	C=O stretching in conjugation to the aromatic ring
1 605-1600	Aromatic ring vibrations
1 515-1 505	Aromatic ring vibrations
1 470-1 460	C-H deformations (asymmetric)
1 430-1 425	Aromatic ring vibrations
1 370-1 365	C-H deformations (symmetric)
1 330-1 325	Syringyl ring breathing
1 270-1 275	Guaiacyl ring breathing
1 085-1030	C-H, C-O deformations

Another feature observed in the FTIR spectra for the fulvic acid fractions is the decrease of the band at  $\sim 1036\text{ cm}^{-1}$  in the low molecular size fractions. This band is usually assigned to the C-O stretching of carbohydrate structures or aromatic C-H deformation. In this case, Duarte et al. (2003) argued that this band could be due to the stretching of carbohydrate structures as the intensities of the bands at  $\sim 1518$  and  $1114\text{ cm}^{-1}$  suggest a lower degree of

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aromaticity in the higher size fractions. Therefore, the intensity of the band at  $\sim 1036\text{ cm}^{-1}$  suggests that the highest size fractions contain more carbohydrate structures.

The interpretation of FTIR for humic substances is often complex. This is due to severe overlapping of characteristic spectral features caused by the complexity and poly-functionality of the humic substance molecule. A better access to the chemical and structural properties of humic substances is often achieved by coupling of spectroscopic methods with chromatography (HPLC) (Abbt-Braun, 1993).

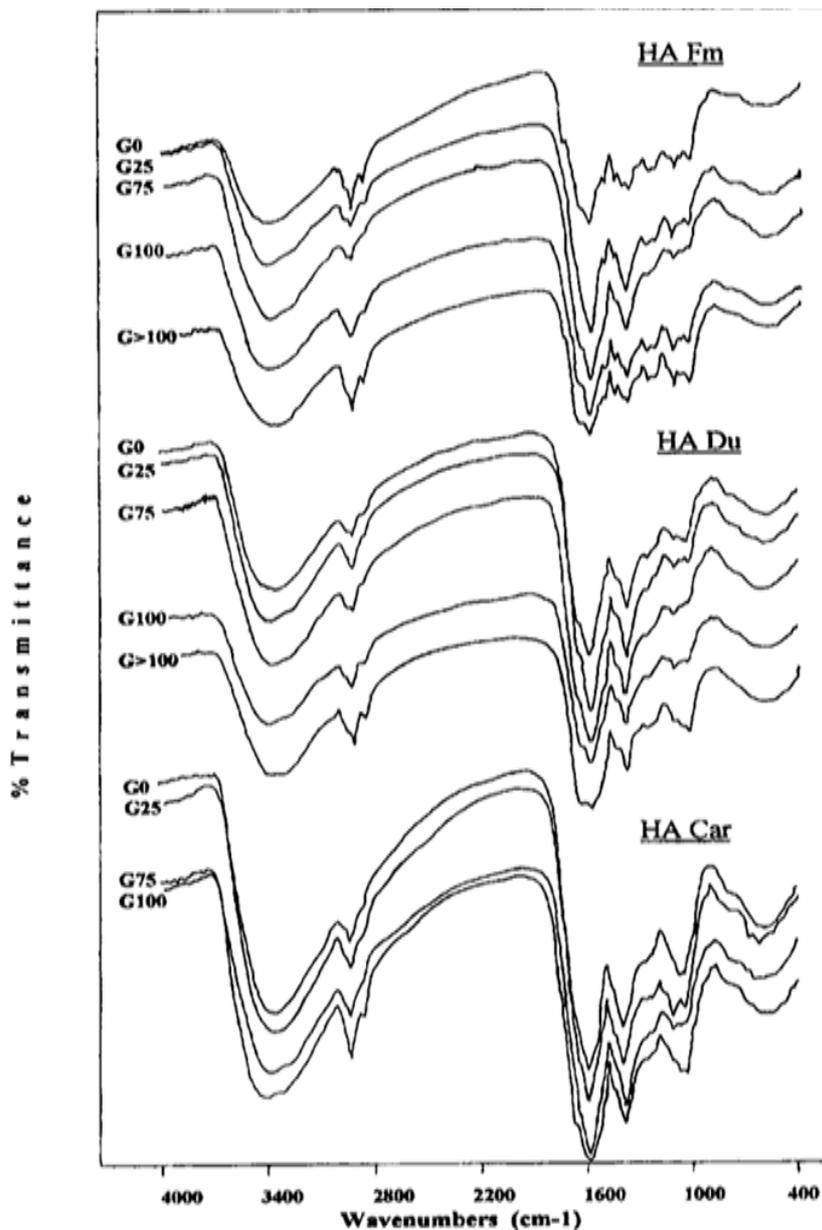


Figure 2.16: FTIR spectra of the unfractionated and fractionated humic acids (Outmane et al., 2000).

### 2.8.3 Chromatographic methods

High Performance Liquid Chromatography (HPLC) is one of the chromatographic methods most used in the analysis of humic substances. Using HPLC, humic substances can be separated into fractions of different structural features. The HPLC technique can also be helpful in comparing humic fractions derived from different sources. Structural information can be obtained by relying on the extent of separation from the chromatographic column (Woelki et al., 1997). Aquatic fulvic acid is reported to be recovered or separated up to 70% using reverse phase chromatography (Saleh et al., 1989). Both Frimmel et al. (1992) and Saleh et al. (1989) observed maximum recoveries of 200-300% for both aquatic and soil humic substances. This implies that they recovered two to three times more of fulvic fractions than available in the starting material. In their process of producing fulvic acid from aquatic and soil humic substances they synthesized more fulvic acid.

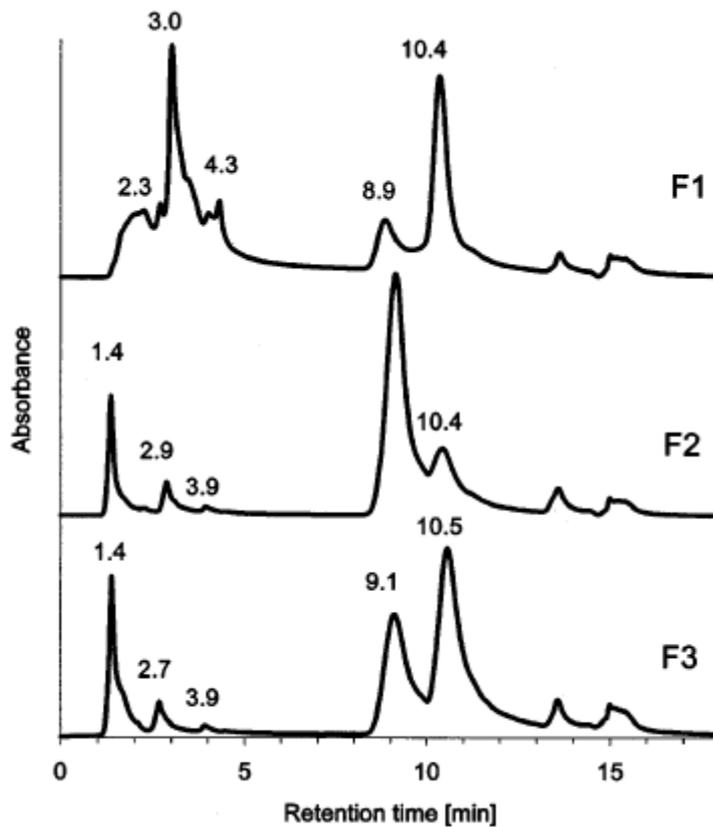
In most chromatographic studies of humic substances, reverse phase columns had a pore size of 100Å (Woelki et al., 1997). The separation of humic substances in reverse phase chromatography is mainly based on adsorption process, although it can also be influenced by exclusion phenomena. Excluded components of the analyte do not penetrate the pores though the solvents molecules do, hence the excluded components are eluted at retention times shorter than the dead time  $t_0$ . For this reason, an exclusion peak whose molecular size is too large to penetrate the pores of the stationary phase is observed on reverse phase chromatography during elution of components. Woelki et al. (1997) showed that humic substances in the native form expose their hydrophilic parts in the aqueous solvent whereas their hydrophobic parts are accumulated to the internal complexes. Separation is also based on the differences in hydrophobicity property where the most hydrophilic fractions are eluted first while the most hydrophobic features are eluted last as schematically illustrated in Figure 2.17.

According to the studies conducted by Woelki et al. (1997), the structure of humic substances consists of fragments (F1, F2, and F3) of medium molecular weight. In addition, Sontheimer et al. (1990) discussed the importance of tertiary structures in aquatic humic substances. For humic substances of similar primary structure, all these authors observed a different behavior in adsorption processes. They attributed this behavior to different secondary and tertiary structures.

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**2.8.4 Elemental analysis**

Elemental analysis is the commonly used technique in characterizing humic substances (Rice and MacCarthy, 1991). The technique allows the determination of C, H, O, N and S, etc. as well as elemental ratios such as C/N ratio, etc. Using this method, the elemental composition and differences between humic substances of various sources after chemical treatment can be examined (Kordel et al., 1997).



**Figure 2.17: HPLC separations of the Na-HA fractions F1, F2 and F3 (Woelki et. al., 1997).**

Since chemical analysis is the cornerstone of all chemical inquiry, elemental analysis can be used to evaluate the effect of the preparation method on the purity of humic substances. Elemental composition is one of the fundamental approaches used in describing the geochemistry of the isolated substances and is often used as an indicator of the unique nature of a given humic substance.

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Table 2.5: Elemental analysis of humic acids extracted from soils from widely differing climatic zones (Schnitzer, and Khan, 1978).

Element (%)	Arctic	Cool temperature		Subtropical	Tropical
		Acid soils	Neutral soils		
C	56.2	53.8-58.7	55.7-56.7	53.6-55.0	54.4-54.9
H	6.2	3.2-5.8	4.4-5.5	4.4-5.0	4.8-5.6
N	4.3	0.8-2.4	4.5-5.0	3.3-4.6	4.1-5.5
S	0.5	0.1-0.5	0.6-0.9	0.8-1.5	0.6-0.8
O	32.8	35.4-38.3	32.7-34.7	34.8-36.3	34.1-35.2

Table 2.5 shows data for elemental analysis of humic acids for an experiment conducted by Schnitzer (1986). The results presented in these tables were in ranges, as more than one data set was available in a given range. From this table it was observed that humic acids contain both C and O as major elements. Schnitzer (1986) found that this behavior was indicative of complexity of the structures of humic acids.

## 2.9 SUMMARY

The study of humic substances shows that they constitute bulk organic matter in the soil and sediments. Their formation begins with the humification or decomposition of organic matter by microbial activity. In nature, lignin and cellulosic materials are the main components from which humic substances are formed. However, chemical processes in the pulp industry also transform lignin in the parent tissue of wood material into fractions of humic substances. The extraction of humic substances using alkaline solution still remains the most used method, although it is thought to cause a negative effect on the structure of humic material. The method used for the extraction of humic substances mainly depends on the nature of humic material in question.

The chemical structure and reactions of humic substances has been the subject of numerous studies, yet there remain many grey areas that require further rigorous investigations. Studies show that the structure of these substances is related to the biological components from which they are formed. The classical differentiation of organic matter into insoluble humins, humic and fulvic acids is the first step in its classification process. However, recent availability of sophisticated analytical techniques, such as FTIR and chromatographic methods, has assisted in elucidating important information on the building blocks that make up humic materials. Besides these milestone developments in this field of study, it is unclear how the building blocks align and the forms of structural arrangements they produce.

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The application of chemical fertilizers to soils and plants to improve food production is increasing at an alarming rate. This can result in an increase in toxic amounts of chemicals found in the soil. By providing large external surfaces for adsorption and firmer retention in internal spaces humic substances react with these chemicals therefore accumulating large amounts of toxins found in the soil (Schnitzer and Khan, 1978). Unfortunately, no studies have been done to find out what happens when the adsorption capacities of humic substances are exhausted or the feasible outcomes in cases where unknown reactions occur suddenly. These two questions should be of concern to environmentalists as the world struggles to maintain or even increase agricultural productivity to feed an exponentially growing population.

Based on this review it appears there is potential to introduce the use of humic substances in South African agriculture on a large scale. This is because humic substances are readily available particularly in black liquor from the pulp and paper industry. However, because of the chemical nature of pulp processes, humic substances isolated from black liquor need to be extracted with great caution to avoid exacerbating environmental problems such as soil salination, which may in turn hinder plant growth. For instance, high levels of sodium chloride (NaCl) may obviously contribute to high levels of Na<sup>+</sup> in the soil which may eventually result into high clay content causing lack of aeration in soil particles. This condition may resist plant root penetration. Excess amount of Na<sup>+</sup> in the soil will neutralize the negative electrical charges that normally cause clay particles to repel each other. Therefore, it is important to monitor the salt content of humic substances before they can be recommended for use in agricultural soils.

## **CHAPTER 3. MATERIALS AND METHODS**

### **3.1 INTRODUCTION**

Research activities for this study were conducted in four phases as follows:

**Phase I:** testing the hypothesis that black liquor is a potential source for the extraction of humic acids. Comparing the yields and E4/E6 ratios of humic acid products from black liquor to humic acid products produced from other possible sources selected randomly.

**Phase II:** developing a suitable method for the extraction of humic acids from black liquor.

**Phase III:** evaluating the effect of humic acids produced on plant growth.

**Phase IV:** develop a possible process flow for the large-scale production of humic acids based on the method of extraction and black liquor material from which a better quantity and quality of humic acids was produced.

### **3.2 EXPERIMENTS**

This chapter provides information on the methods that were used to select suitable humic substances for the extraction of humic acids. Procedures, materials, and analytical methods used to determine suitable extraction methods and quality of humic acids produced are outlined. This section also reflects experiments performed to determine effect of humic acids on plant growth. Finally, a framework for the possible process flow for the production of humic acids at large-scale is presented. A list of chemical and materials used to carry out experiments and tests is given in Appendix 8.3, Table 8.3.1.

### **3.3 TESTING THE HYPOTHESIS THAT BLACK LIQUOR IS A POTENTIAL SOURCE OF HUMIC ACIDS AND A COMPARISON OF BLACK LIQUOR TO OTHER SOURCES.**

A variety of raw materials was randomly selected and treated using the acid-base precipitation method to extract humic acids (IHSS, 2005). These materials are abbreviated as 5-week old compost (C5W), 6-week old compost (C6W), Blaauwklippen compost no.1 (CB1), Blaauwklippen compost no. 2 (CB2), commercial compost (C\_com), “goodie water” (GW), and black liquor (BL) where:

C5W: is a compost sample collected from 5-week composted material generated by composting cuttings, horse manure, straw, grass, vegetables and grape waste C6W: is a compost sample collected from 6-week old composted material comprising of a mixture of cuttings and grass;

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CB1: is a compost material generated by mixing and composting chicken manure, urea and straw at the temperature of 25°C for 13 days (Figure 3.1);

CB2: is a compost material that remains as a by-product from mushroom production and is valueless to the mushroom farm (Figure 3.2);

C\_com: is a sample of commercial compost obtained from an Agrishop just outside Stellenbosch;

GW: is runoff water from composting of CB1; and

BL: is black liquor from pulp and paper industry.

Materials were selected on the bases of their availability, suitability and accessibility for the long term results of this work which included production of humic acids on a large scale. The results of this phase are based on quantity and E4/E6 ratios (see glossary for definition) of the product from each material. Finally, the hypothesis that BL is a potential source for the production of humic acids was tested and confirmed by comparing results to those of other potential sources selected.

### 3.3.1 Sampling

Raw materials containing humic substances were sampled at random to avoid statistical bias. Samples of raw materials collected from the source were handled based on their physical state for example effluent materials were sampled using 5 l plastic containers/bottles with a stopper whereas compost materials were sampled using 5 l plastic bags.

In the laboratory, compost materials were stored in a refrigerator at less than 4°C to minimize microbial activity before the experiments were conducted. After one month five compost material samples were dried in an oven overnight at 60°C to eliminate moisture without altering the organic carbon content of each sample. The removal of moisture was observed by loss of weight during the drying period. Samples were weighed after being cooled to room temperature in a desiccator containing silica gel to avoid reabsorption of moisture from the atmosphere. The loss of moisture varied according to the type of sample material. A fume mask and rubber gloves were used when handling the samples in the laboratory because of the strong smell from the samples due to their state of decomposition or chemical content. It was also necessary to use a fume mask and rubber gloves to avoid inhalation and touching of toxic chemicals produced by decomposition of the samples or industrial chemicals from industrial processes. This was most important for the black liquor material.

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Before subsequent experiments were conducted, dried compost samples were pulverized using a mortar and a grinding crucible to create large surface area to encourage proper penetration of the extraction solution. Effluent samples were filtered using 0.45  $\mu\text{m}$  membrane filter before the extraction process was conducted, allowing only dissolved organic carbon to pass through and then centrifuged for complete removal of the suspended solids. A brief description on how each sample was collected is given in sections 3.3.2 – 3.3.4.

### 3.3.2 Compost samples

Compost materials, C5W and C6W were collected from Reliance Compost available at Spier (Pty) Ltd just outside Stellenbosch. The samples were obtained by open-air composting in windrows. Two other compost sample materials CB1 and CB2 (Figures 3.1 and 3.2) were collected from Medallion Mushroom farm. Medallion Mushroom farm is a farm growing mushrooms for commercial purposes at Blaauwklippen situated about 3 km south of Stellenbosch. The compost presented in Figure 3.1 (CB1) was used as a substrate in the production of mushrooms. During the process of composting, CB1 is overturned from time-to-time to encourage uniformity in microbial activity. The compost (CB2) presented in Figure 3.2 is a mixture of Turf, CB1 and some mushroom remains. Turf is the dark coloured soil delivered from Johannesburg. This soil is overlaid on top of CB1 after inoculating CB1 with the mushroom spawn. Due to its clay texture, this soil is used to maintain moisture without inhibiting the protrusion of mushroom buds from the substrate during mushroom production. Currently, CB2 is donated to the surrounding farms for gardening and farming purposes.



**Figure 3.1: CB1- compost from urea, chicken manure and straw for mushroom production at Medallion mushroom farm.**

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**Figure 3.2: CB2 – a by-product compost produced after mushroom production from Medallion Mushroom farm.**

Lastly, a sample of a commercial compost sold as a product of Omega Misstowwe BK Stellenbosch was bought from an Agrishop. This compost is commonly referred to as the enriched fertilizer [5:3:6 (14); 55 g/kg N, 27 g/kg P, 60 g/kg K; Misstof Group fertiliser] with 3.5% content of humic acids (manufacturers specifications).

### 3.3.3 Effluent samples

A sample of runoff water expressed as “Goodie Water (GW)” was collected from Medallion Mushroom farm. During the preparation of compost (CB1) at Medallion, water is added from time-to-time to the blocks of straw before they are open and mixed with chicken manure. “Goodie Water” is the water that drains out from the straw into a small recycling pond during the composting process. This sample was collected within a period of one day.

A dark coloured, sample of black of liquor (BL) was received from Sappi Kraft. The sample was received in a tightly sealed plastic bottle as supplied by Sappi. This sample was sent by courier and arrived at the University within a period of one full day. Based on the length of time that took the sample to arrive at the University it was assumed that the sample was still in good condition when it arrived in the laboratory. Due to its high concentration the sample was diluted with 0.1 M NaOH before experiments were conducted.

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**3.3.4 Humic (Na-HA) acid standard sample**

A sample of humic acid standard (Na-HA) from Fluka, Switzerland said to be isolated from natural dam water and to express a molecular weight (MW) of approximately 1200 g/mol (According to Professor Swart) was received. Humic acid from Fluka has been used as a reference for quantification in previous studies (Rigol et al., 1998). Both Na-HA were used in this study for finger-printing purposes. Even though this sample was not meant to give an ideal comparison of humic acids produced in this study, its inclusion would provide an idea of the quality of humic acids produced.

**3.3.5 Commercial humic acid (HA\_com)**

A sample of commercial humic acid was received from Germany with the help of Chris Vermeulen. However, no specifications were provided with regard to its chemical composition and source of production.

**3.4 EXTRACTION OF HUMIC FROM COMPOST SAMPLES**

The humic acids available in the raw materials were extracted by basic acid-precipitation method which follows the principle of acid-base reaction. Samples from compost materials were first treated with sodium hydroxide (NaOH) to extract the humic substances from the raw material before the solution was treated with an acid to precipitate humic acids. The procedure that was followed is defined in the subsections that follow:

**3.4.1 Compost samples**

A 10 g sample from each compost material was weighed and transferred into a 200 ml beaker and treated with 0.1 M sodium hydroxide (NaOH) to recover the soluble humic substances from the raw material. A solvent of 50 ml of 0.1 M NaOH solution was added to 10 g sample material in a beaker with some means of agitation (stirrer bar). At the end of 1 hr the mixture was transferred into centrifuge tubes to separate the solution from the residue. Centrifugation was carried out at  $1 \times 10^{-7}$  g for 30 minutes. The residue was treated twice with 0.1 M NaOH and finally once with distilled water and centrifuged to assume complete recovery of humic acids. The solution recovered was filtered through a 0.45  $\mu\text{m}$  membrane filter by using a Buckner funnel and a suction flask to remove suspended solids. The solution was then transferred into a 300 ml glass bottle with a stopper that had two holes. In one hole of the stopper was fitted a pipette connected to a tube extending from the nitrogen gas cylinder. The other opening was an outlet for the escape gas formed during reaction. Through the outlet,

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concentrated HCl was added drop-by-drop timeously using a pipette to adjust the pH of the solution to a pH less than pH 2. In the meantime the solution was continuously stirred under a nitrogen atmosphere to precipitate the humic acid whilst purging inorganic carbons like CO<sub>2</sub> and bicarbonates. After precipitation the solution was allowed to stand for about 4 hrs to allow total precipitation of humic acid. Subsequently, the precipitate was transferred into centrifuge tubes for centrifugation at  $10 * 10^{-7}$  g for 30 min to recover the humic acid sludge from the remaining solution. The humic acid sludge recovered was re-dissolved in 0.1 M NaOH solution and re-precipitated with concentrated HCl in an attempt to remove impurities. This step was repeated twice to allow more removal of impurities from the humic acid sludge. The humic acid sludge in centrifuge tubes was transferred into a vacuum oven at a temperature of 55°C for overnight to avoid loss of organic carbon through decomposition at higher temperatures. The product of humic acid was weighed, pulverized and stored in a vial for further analysis. The remaining solution was discarded.

**3.4.2 Extraction of humic acids from effluent samples**

The pH of 100 ml “Goodie Water” was adjusted to a pH of less than 2 using concentrated HCl to precipitate the humic acid. The precipitation process was conducted under nitrogen atmosphere to purge inorganic carbon in the form of CO<sub>2</sub> and bicarbonates from the solution. The solution with the precipitate (humic acid sludge) was transferred into centrifuge tubes and centrifuged at  $1 * 10^{-7}$  g for 30min to recover the humic acid sludge from the solution. The sludge was re-dissolved in 0.1 M solution of sodium hydroxide (NaOH), re-precipitated by HCl and centrifuged to rid it off impurities including salts. This stage was repeated twice to maximize the removal of impurities. The humic acid sludge in centrifuge tubes was oven-dried at 55°C under vacuum keeping the organic carbon content of the sample stable. Finally, the sample of dried humic acid was pulverized using a mortar and a porcelain dish, weighed and stored in a vial.

The same precipitation method as in effluent samples was used for the extraction of humic acids from black liquor received from Sappi Kraft. However, prior to extraction 10 ml of black liquor (BL) was diluted to 100 ml with 0.1 M NaOH due to its high concentration which made it difficult to work with this material at its raw state. Calculations made to compare yields of effluent samples to those of compost samples are shown in Appendix 8.1.2.

### 3.5 FORMULATION OF EXTRACTION METHODS

For the second phase of research activities four methods were developed, namely humic acid precipitation method (HAp), lignin extraction/humic acid precipitation method (LHAp), humic acid precipitation/acid treatment method (HApA), lignin extraction/humic acid precipitation/acid treatment method (LHApA). The development of these methods was based on the extraction method recommended by International Humic Substances Society (IHSS). According to IHSS the main procedure in the extraction of humic acids is in the precipitation of humic substances. These methods were formulated based on the composition of black liquor which was selected as a potential raw material for the extraction and production of humic acids in South Africa. The formulation of these methods was meant to determine the effect of lignin fractions present in black liquor and the effect of black liquor material (hardwood, softwood or bagasse) from which black liquor originated on the quantity and quality of humic acids.

#### 3.5.1 Sampling

The findings of Phase I confirmed the hypothesis that black liquor is a potential source of humic acids in South Africa. Due to these findings more samples of black liquor were obtained from pulp and paper companies such as Mondi Business Paper and Mondi Packaging Felixton (see Table 3.1 and Figure 3.3). These samples were collected from different digester units e.g. the *Eucalyptus*, pine and bagasse digester units i.e. hardwood, softwood and bagasse materials. Samples were collected in 25 l containers and sent by surface to the University of Stellenbosch, Chemical Engineering laboratory. Upon arrival in the laboratory the samples were subsequently stored at room temperature before experiments were conducted. No major alteration and effect on the experimental results were expected due to the manner in which these samples were sent. Figure 3.3 shows 25 liter samples of different black liquor materials. The quantity of humic acids available in each effluent depended on the source of each effluent material.

Table 3.1: Black liquor samples received from pulp and paper companies.

Company	Liquor	Sampling point	pH
Mondi	<i>Eucalyptus</i>		12.78
	Pine		12.90
Felixton	Bagasse		7.9

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**Figure 3.3: Black liquor samples from Mondi Business Paper and Mondi Packaging Felixton.**

The procedures that were followed in each method are discussed below starting with the list of materials that were used.

### **3.5.2 Humic acid precipitation (HAp) method**

One liter of black liquor solution with pH greater than pH 12 was filtered through a 0.45  $\mu\text{m}$  membrane filter to eliminate any suspended solids and transferred into a 5 l vessel with some means of agitations. Immediately, concentrated HCl was added carefully to avoid spillages as the mixture started to thicken out and thinned out again as the precipitate began to form. This procedure was conducted under nitrogen atmosphere. Concentrated HCl was added until the pH of the mixture was less than pH 2, and then the stirring of the mixture was stopped. The mixture was allowed to stand for 3 to 4 hours in a closed container. At the end of 4 hrs the mixture was transferred into centrifuge tubes and centrifuged at the speed of  $1 * 10^{-7}$  g for 15 min. The precipitate settled at the bottom of the centrifuge and was recovered by discarding the solution. The humic acid sludge (precipitate) was re-dissolved in 0.1 M NaOH, re-precipitated and centrifuged to remove impurities. This step was repeated twice to ensure maximal removal of impurities. The centrifuge tubes with humic acid sludge were transferred into a vacuum oven at a temperature of 55°C overnight to dry out the humic acid sludge. The humic acid was then pulverized, weighed and stored in small vials and kept for further analysis.

### **3.5.3 Lignin extraction/humic acid precipitation method (LHAp)**

The LHAp method was divided into two steps. The first step was the recovery of lignin fractions from the black liquor and the final step was the extraction of humic acids from the

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remaining solution after lignin fractions have been removed. These steps are defined as follows:

**3.5.3.1. Extraction of Lignin fractions**

The lignin extraction method was conducted based on the type of black liquor i.e. black liquor produced from a Kraft process. The extraction of lignin in this study was modeled after the procedure described by Holsopple et al. (2004) and is described as follows:

***Kraft lignin extraction:*** one liter of Kraft black liquor was placed into a 4 liter vessel with an overhead stirrer. Then 250 ml of water was added into the same vessel with the black liquor. Mesityl oxide (175 ml) (4-methyl-3-penten-2-one solvent) was also added. As the mixture was stirred, 62.5 ml of 80% acetic acid was added carefully and stirred for further 10 minutes. The mixture started to thicken then thinned out again. At this time, additional 75 ml of 80% acetic acid was added until the mixture was acidic. The stirring was stopped 10 minutes after the addition of acetic acid. The mixture was immediately transferred into a 5 liter separating vessel and allowed to stand for 30 minutes. The lignin solvent layer settled on top of the vessel. The solution at the bottom of the vessel was drained out and the lignin layer transferred into a porcelain dish, thereafter to the vacuum oven at a temperature of 55°C to dry out the solvent. After two days the lignin was removed from the oven and placed in a desiccator to allow the dried material to cool down to room temperature. The next day the lignin was pulverized, weighed and stored in small vials for further analysis.

**3.5.3.2. Extraction of humic acids**

The solution recovered after lignin extraction was evaporated in an oven under vacuum conditions to remove the solvent. Possibly, this process could be better if spray-drying was used however, because there was no spray drier in our department the solution was evaporated under vacuum conditions. The dried material was then dissolved in 0.1 M NaOH by heating the mixture to a boiling point whilst continuously stirring. This process was conducted in a fume cupboard. After four hours the solution mixture was left to cool down to room temperature and then filtered through a 0.45 µm membrane filter paper. The solution was then transferred into a bottle with a two holed cock stopper. One of the holes was used as a nitrogen gas inlet and the other as an escape gas outlet as well as an inlet for the pipette which was used to add HCl drop-by-drop into the stirred solution mixture to adjust the pH to less than 2. The stopper would be removed quickly from timeously to monitor the pH using a pH meter. When the pH was less than 2 a precipitate was formed and the solution was allowed to

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stand for about 3 hours to allow the reaction to complete. After three hours the solution mixture was transferred into centrifuge tubes and centrifuged at a speed of  $1 \times 10^{-7}$  g for 15 min. From here the same procedure as in HAp was followed starting from this step as indicated in humic acid precipitation method.

### **3.2.2.4 Humic acid precipitation/acid treatment method (HApA)**

The procedure in this method was the same as in HAp method except that in HApA method the humic acid sludge recovered after centrifugation was not re-dissolved in 0.1 M NaOH but treated three times with 30% dilute HCl. The sludge was treated by shaking it with HCl and centrifuged to recover the sludge. This step was also meant to remove impurities from the humic acid.

### **3.2.2.5 Lignin extraction/humic acid/acid treatment method (LHApA)**

LHApA method was also the same as LHAp method except that in this method the humic acid sludge recovered after centrifugation was also treated with 30% dilute HCl as in HApA method.

## **3.6 QUALITATIVE ANALYSIS**

After extraction, the samples were analyzed qualitatively. For this, purpose a number of methods namely Elemental analysis, UV-VIS, FTIR and HPLC were selected based on their availability and suitability (as determined in literature) for the analysis of humic substances. Experimental procedures for these methods are discussed in the following sections. A comparison between spectra helped in identifying similar IR features regardless of the nature of humic acid and the method of extraction used. The results from this method were able to complement each other by confirming observations as determined by another method.

### **3.6.1 Elemental analysis**

Carbon (C) and nitrogen (N) composition of the extracted humic acids including humic acids standard (Na-HA) and lignin was analyzed using EuroVector Elemental Analyzer (EA) from Department of Soil Sciences, University of Stellenbosch, while the sulphur content was determined by a Leco CS200. The ash content of all the samples was obtained by combusting a known amount of each sample ( $M_0$ ) in the Gallenkamp Muffle Furnace available in the Department of Wood Sciences, University of Stellenbosch. Ash content analysis was determined by putting each sample in an oven and gradually increasing the temperature from 100°C to 450°C. The mass difference between the original weight of the sample before

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combustion ( $M_o$ ) and the weight of the sample after combustion ( $M_a$ ) was regarded as the ash content.

### 3.6.2 Ultraviolet Visible (UV-VIS) Spectroscopy

UV-VIS spectra were used to elucidate structural features of humic acids, to determine the degree of aromaticity and molecular size of the humic acids. A solution of humic acid samples including Na-HA and lignin samples was prepared in 0.01 M NaOH for each sample material. The solution was prepared by dissolving a small amount (about an edge of the tip of a spatula) of the sample in 50 ml 0.01 M NaOH solution. The solution was thoroughly shaken and then left overnight to allow enough time for the material to dissolve. The next morning the solution was filtered through 0.45  $\mu\text{m}$  membrane filter paper to remove possible suspended solids that would interfere with the analysis. The ultraviolet visible spectroscopy of lignin, humic acids was carried out using the Cary 1E spectrophotometer, in a 1 cm quartz cell. The spectra were recorded from 700 to 200 nm. A solution of 0.01 M NaOH was used as a blank. The E4/E6 ratios were determined by taking the ratio of the absorbance at 465 and 665 nm from the UV-VIS spectroscopy data table.

### 3.6.3 Fourier Transform Infrared (FTIR) Spectroscopy

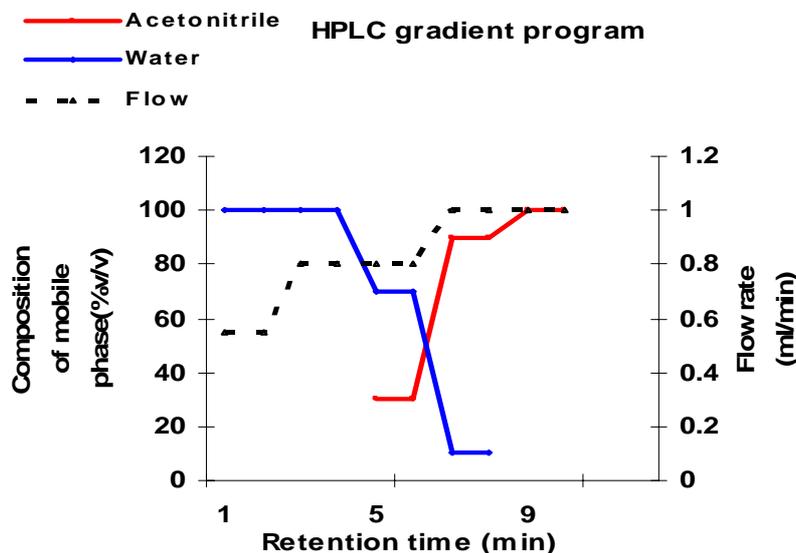
The FTIR spectra were recorded on a Nexus FTIR spectroscopy with thermo Nicolet sample processor using KBr-pellets and NaCl. All samples were finely pulverized together with spectroscopic grade KBr and pressed into pellets. The sample/KBr ratio was estimated at 1/300 mg (Sanchez-Monedero et al, 2002). The sample compartment was purged with nitrogen for 10 minutes to drive off oxygen before the sample analysis was conducted or each spectrum was recorded. The spectra resolution of 4  $\text{cm}^{-1}$  was obtained by averaging 100 scans. All spectra were scanned in the wavelength range of 4000 to 500  $\text{cm}^{-1}$ . The excitation lamp spectral profile and temporal intensity variations were automatically corrected during the recording of spectral peaks.

### 3.6.3 High Performance Liquid Chromatography

Humic acid products from black liquor, standard humic acid (Na-HA) and commercial humic acid produced from Leonardite were fractionated using HPLC. HPLC separations were determined using HPLC TSP system, P2000 pump, Auto-sampler AS3000 equipped with a UV 1000 at 254 nm detector. A commercial pre-packed analytical HPLC column, EC 250/4 Nucleosil 1000-7 C18, was used (Separations South Africa; an agent of Macherey-Nagel). All

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injections were done at 100  $\mu$ l volume (0.1 ml humic acid sample solutions). The rate of injection is presented in Figure 3.4. Samples of humic acid including Na-HA standard were dissolved in 0.01 M NaOH, and were filtered before injection just as they were prepared for UV-VIS spec. The stepwise gradient program for the separation of humic acids followed in this study is presented in Figure 3.3.



**Figure 3.4: Stepwise gradient program for the separation of humic acids.**

Figure 3.4 outlines the graphical presentation of the method of separation that was used to separate humic using chromatographic separation. The composition of the solvent (acetonitrile, water) and the elution program (isocratic, gradient and flow) varied.

### 3.7 EVALUATING THE EFFECT OF HUMIC ACIDS ON PLANT GROWTH FOR HUMIC ACIDS PRODUCED BY THE TWO METHODS SHOWING HIGHER YIELD EFFICIENCY.

Humic acids produced by the two the methods showing higher extraction efficiency of humic acids (76 HAp to 89 % HApA *Eucalyptus* and 28 HAp to 60 % HApA pine liquor) compared to the other two methods were investigated for their effect on plant growth. A mixture of these humic acids and micronutrients was prepared to investigate the effect of humic acids produced by these methods on plants growth. This effect was evaluated relative to the control that includes plants grown on clean soil (blank) and plants growth on micronutrients alone. A mastered seed (*Brassica nigra*) chosen based on its short growth period and ease of height measurement. The growth rate data of the plant was collected and tabulated for simple statistical analysis.

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The humic acids applied were produced from Kraft black liquor received from Mondi Business Paper and black liquor received from Mondi Packaging Felixton. Data collection on the growth rate of mustard plants was conducted and recorded over a period of four weeks. The experiment was carried out inside a house in an area that had sufficient natural light and room temperature.

### 3.7.1 Selection of plant growth materials

**Soil type:** To avoid unnecessary contaminations a sample of pre-treated sand, grade twelve from Consol limited industrial minerals was used as a medium for growing the plants. This was done on the bases that this soil is pre-treated and assumed to contain no nutrients that would interfere with the test being conducted.

**Micronutrients:** chemicals containing micronutrients i.e. nitrogen, phosphorus and potassium (N, P and K) were received from the laboratory. These are commercially available chemicals. Generally, commercially available fertilisers provide N, P and K in the balance desired for crop production. However, in this study Table 8.3.2 and Table 8.3.3 in Appendix 8.3 were used as a quick guide to determine the required amounts for each of these micronutrients and to prepare desired concentrations. For nitrogen in this study ammonium sulphate  $[(\text{NH}_4)_2\text{SO}_4]$  was used as a source of N whereas for P and K, potassium phosphate  $[\text{H}_2\text{KO}_4\text{P}]$  was used.

### 3.7.2 Preparation of a fertilizer solution

A solution of these nutrients was prepared in 720 ml solution of 0.01 M NaOH in such a way that the solution provided the concentration of these elements in a (15:16:16.3) ratio. This is the ratio recommended generally for crop production (Fertilizing Greenhouse Crops, 2006). Table 3.2 indicates how this solution was prepared based on Tables 8.3.2 and 8.3.3 in Appendix 8.3.

Table 3.2: Measurement of micronutrients used in the preparation of humic acid solution for the plant trial test.

<b>Source of element</b>	<b>Amount/720ml</b>	<b>N</b>	<b>P</b>	<b>K</b>
Ammonium sulphate	0.03g	15	-	-
Potassium phosphate	0.23g	-	16	16.3

### 3.7.3 Determination of Soil pH

The pH of the soil was determined by adding 20 ml of water into 100 g of soil and mixed thoroughly. The mixture was filtered and the pH of the solution was tested and found to be pH 6.7.

### 3.7.4 Determination of the water holding capacity of the soil

The water holding capacity of the soil was determined by adding tap water to 100 g of pre-sieved soil with pre-determined mass in a beaker. The saturation of the soil was observed by glistening of the soil surface. The beaker was then allowed to stand for a few minutes and a few drops of water were added until saturation was complete. The beaker was then weighed to determine the amount of water used to achieve saturation. The saturation of 100 g of soil was found to be 18 ml tap water.

### 3.7.5 Preparation of humic acid solution

A solution of humic acid was prepared by adding 0.03 g ammonium sulphate, 0.23 g potassium phosphate and 2 g humic acid into 720 ml 0.01 NaOH. The micronutrients composition of this solution was given as 15:16:16.3 (N: P: K) as indicated in Table 3.2.

### 3.7.6 Experimental

Four kilograms sandy soil was added into ten 3000 cm<sup>3</sup> (7 cm diameter) pot. This was followed by addition of a solution containing humic acids and micronutrients with nitrogen, potassium and phosphorus as micronutrients of interest. Humic acids produced by both HAp and HApA methods from all the black liquor materials were investigated for their effect on plant growth: The following materials were also used for control purposes HA\_com, blank, and micronutrients.

The dosage of nitrogen element was determined to be a function of the surface area of the pot. To each pot, 12 mustard seeds were planted around the surface area of the pot with equal spacing in-between the seeds. The solution mixture was added only during the planting period; however, the pots were continuously watered with ordinary tap water after every two or three days with the amount of water depending on the rate of evaporation. The effect of humic acid was determined by measuring the growth rate of plant from seed germination to the plant height after four weeks. The height of the plants was measured every week and the average

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recorded in the table for data analysis. At the end of the four weeks a simple statistical analysis was conducted and the results are presented in the form of a table and graphs.

**3.8 DEVELOPING A CONCEPTUAL PROCESS FLOW**

A conceptual process for the large-scale production of humic acid was developed. This process was developed based on the recommended method of production of humic acids and was adapted based on the material that gave the better quality and the highest quantity of humic acids products. The process-outline mimics activities carried out in the laboratory and is meant to provide a guideline for the development and research for a large-scale production plant. It is by no means providing an ideal design of a production plant. A number of concepts were recognised in the development of this process and amongst these are the following concepts:

- Input output flow
- Specific activities that were carried out in the laboratory.

## CHAPTER 4. RESULTS AND DISCUSSIONS OF PHASE I AND II

### 4.1 INTRODUCTION

This section presents results from the two main sets of experiments reflected in Phases I and II (Chapter 3) conducted in this study to address the following objectives: testing the hypothesis that black liquor is a potential source for the extraction of humic acids and developing a suitable method for the extraction of humic acids from black liquor.

### 4.2 TESTING THE HYPOTHESIS THAT BLACK LIQUOR IS A POTENTIAL SOURCE OF HUMIC ACIDS AND ITS COMPARISON TO OTHER SOURCES.

All seven materials, C5W, C6W, CB1, CB2, GW, BL, and C\_com (see glossary for the definition of terms) investigated for testing the hypothesis that BL is a potential source for the extraction of humic acids with extraction conducted in three replicates showed some potential of BL as possible source of humic acids. The order of production yields of humic acids from these materials was  $GW < C\_com < C6W < CB2 < CB1 < C5W < BL$  statistically significant. Oven dried weights of the samples before extractions are shown in Table 4.1 and results are presented in Table 4.2.

The yield results are presented in terms of percentages per mass or volume of material used depending on whether the material was effluent or compost in nature. Volume of solvent for extraction process also varied with nature of material.

Table 4.1: Loss of moisture from compost samples after oven drying.

Sample	Initial weight (g) (before oven drying)	Final weight (g) (after oven drying)
CB1	100	32
CB2	100	67
C5W	100	90
C6W	100	91
C_com	100	98

The yields of humic acids were calculated and compared as shown in Appendix 8.1.2.

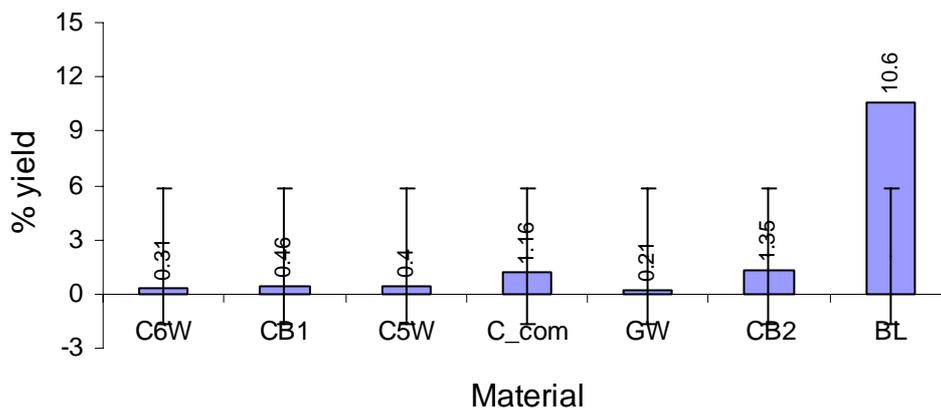
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Table 4.2: Yields and E4/E6 ratios results for the production of humic acids.

Material	Yields %	E4/E6 – HA 3 – 5.9
C6W	0.31 ± 0.05	5.49 ± 0.32
CB1	0.46 ± 0.05	4.71 ± 0.65
C5W	0.40 ± 0.04	6.25 ± 0.93
C_com	1.16 ± 0.02	4.26
GW	0.21 ± 0.03	4.1 ± 0.62
CB2	1.35 ± 0.04	5.73 ± 0.64
<b>BL</b>	<b>10.6 ± 0.16</b>	<b>3.97 ± 0.50</b>

In Table 4.2 BL showed highest content of humic acid (10.6 %, Stdev. 0.16) compared to all the other materials studied. Graphical presentation of percentage yield is shown in Figure 4.1

Testing the hypothesis of BL as a possible sources for large-scale production of HA



**Figure 4.1: A comparison of humic acid yields from black liquor and other sources.**

Figure 4.1 also indicates that the humic acid content of black liquor (BL) was higher (> 79 %) than the humic acid content of the other materials to which BL was compared. The E4/E6 ratio of humic acid produced from black liquor was also observed within the range 3.97 – 5.49 (see Table 4.2), which lies in the region of the accepted range (3 - 5.9) (Kononova, 1966) except for C5W (E4/E6 = 6.25).

#### 4.3 DISCUSSION OF RESULTS FROM HYPOTHESIS TEST

The results provided in Table 4.2 and Figure 4.1 show that black liquor is outstanding in humic acid yields (> 79 %) compared to the other materials assessed in the experiments (< 21 %). The Hypothesis that black liquor (BL) is a possible source of humic acids was thus

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confirmed and BL was selected for further investigations and extractions of humic acids. The humic acid yields produced by the other materials were far too low (more than 10 times lower) than the humic acid yield produced by black liquor (Figure 4.1). However, the E4/E6 ratios which were also one of the bases of testing the hypothesis compared fairly for all the materials except for C5W (Table 4.2). The humic acid produced from black liquor had an acceptable quality in terms of E4/E6 ratio ( $3.97 \pm 0.50$ ) determined using UV-VIS spectroscopy. In the experiment, yields and E4/E6 ratios of humic acids adequately formed the basis for selecting suitable material for the extraction of humic acids.

All the humic materials selected in this study contain substantial amounts of humic acids in their raw state as shown in the yield results (Table 4.2 and Figure 4.1). However, based on the extraction methods used, BL contain increased amounts of humic acids. This study was limited to analysing the selected materials because these were the only materials available in the time of study and were available in sufficient quantities. BL could be a suitable raw material for the production of humic acids at a large scale in South Africa for various reasons, though mostly because of its availability compared to the other materials investigated. It is observed that due to the large and ever growing market in pulp and paper industry, production of black liquor is subsequently increasing exerting more pressure on the pulp and paper industry to find alternative ways of treating and utilising black liquor to reduce potential pollution to the environment. The quantity of humic acids found in black liquor is, however less than the quantities found in sapropel peat, black peat and Leonardite as reflected earlier in Chapter 3, Table 3.1. Besides not producing enough humic acids, sources such as GW, C6W, C5W, CB1, CB2 and C\_com are also not easily accessible, therefore may not be suitable for the large-scale production of humic acids in South Africa. The production of humic acids at a large scale requires that a sustainable raw material be available. Materials like GW, C\_com, CB1, C5W, might not be sustainable for this purpose as their availability depends largely on the mushroom production and may also compete with the commercial sector in the case of C\_com.

Black liquor is currently recycled for energy purposes, in order to avoid competing with the energy recycling. However, depending on the economic impact of producing humic acids from black liquor, production of humic acids from black liquor may take precedence over the use of black liquor as an energy source. On the other hand, large volumes of black liquor produced everyday may create a platform for the production of humic acids to take place at

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once with the use of black liquor as an energy source. Depending on the possible conceptual process flow this may encouraged a better economic impact to the pulp and paper industry resulting from the production of humic acids rather than the use of black liquor for energy production alone. All these are the issues that need to be considered as the large-scale production is being investigated.

### **4.4 FORMULATION OF EXTRACTION METHODS FOR THE RODUCTION OF HUMIC ACIDS FROM BLACK LIQUOR**

Results reflecting the efficiency and suitability of the formulated methods on the extraction of humic acids from black liquor are presented in Tables 4.3, to 4.4 and Figures 4.2, to 4.6. These results are divided into two main parts i.e. yield for each extraction method and analytical results. Analytical results are determined by elemental composition and instrumental analysis of humic acids extracted. Both yield and analytical results reflect the production efficiency of each formulated method (see Chapter 3, Section 3.1) of these products in comparison to the standard humic acid (Na-HA).

### **4.5 QUALITATIVE ANALYSIS OF HUMIC ACIDS FROM BLACK LIQUOR**

The qualitative analytical results (elemental and chemical composition) of humic acids are presented in Tables 4.4 and Figures 4.2, to 4.6.

#### **4.5.1 Elemental Analysis (EA) of humic acids from black liquor**

Results shown in Table 4.4 reflect elemental composition and E4/E6 ratios of humic acids extracted from black liquor. Except for pine liquor material, humic acid extracted from black liquor showed some consistency in the carbon content irrespective of the extraction method. Nitrogen content of humic acids extracted from black liquor materials exhibited some consistence regardless of extraction method. Compared to humic acids from both *Eucalyptus* and pine black liquor materials, lignin in these materials generally showed a low carbon and nitrogen content. Carbon content of Na-HA was relatively lower than that of humic acids extracted from black liquor. However, the opposite was true for nitrogen content except for humic acids extracted from bagasse liquor.

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Table 4.3: Quantitative analysis and E4/E6 ratios of humic acids extracted from black liquor produced from three different materials i.e. *Eucalyptus* (hardwood) pine (softwood) and sugarcane bagasse (Appendix 8.4, Figure 8.4.1).

Method of extraction and humic substance	% Yield	E4/E6 $3 \geq \text{E4/E6} < 6$
HApEuc	6.55 ± 1.60	4.8 ± 0.95
LHApEuc	1.55 ± 0.99	4.9 ± 2.28
HApAEuc	5.14 ± 1.10	<b>2.3 ± 0.134</b>
LHApAEuc	0.54 ± 0.67	4.2 ± 0.48
HApPin	3.50 ± 1.22	4.6 ± 0.88
LHApPin	2.51 ± 2.84	5.9 ± 1.64
HApAPin	3.68 ± 2.53	5.6 ± 0.58
LHApAPin	1.49 ± 0.51	4.9 ± 0.85
HApBag	1.06 ± 0.27	<b>6.9 ± 1.47</b>
HApABag	0.79 ± 0.96	<b>9.0 ± 1.54</b>
Na-HA	-	3.2 ± 0.15
HA_com	-	4.13 ± 0.53
Leuc	3.87	3.71 ± 0.23
Lpin	1.14	7.27 ± 0.51

Figure 4.2 shows that both ash and s content of humic acids (HA) vary most with the type of black liquor material with the variation depending on the type of extraction method. The ash content increases from HA extracted from BLEuc to HA extract from BLPin while the ash content of HA extracted from BLBag is below the ash contents of HA from both BLEuc and BLPin. The s content of HA shows a decreasing trend from hardwood to softwood and from softwood to Bagasse black liquor material.

Regarding the carbon/nitrogen ratios (C/N), a general trend was observed that exhibited higher C/N ratios for HAp and HApA methods than LHAp and LHApA methods for both *Eucalyptus* and pine liquor materials. For bagasse liquor, HAp exhibited higher nitrogen content than HApA.

Relatively, humic acids extracted from bagasse liquor exhibited higher nitrogen content than humic acids extracted from *Eucalyptus* and pine liquors. Na-HA showed lower C/N ratios than humic acids extracted from black liquor. Lignin extracted from *Eucalyptus* liquor exhibited higher C/N ratio than lignin extracted from pine liquor. For both *Eucalyptus* and pine liquor HAp and HApA methods exhibited higher sulphur content than LHAp and LHApA methods (Figure 4.2). Humic acids extracted from bagasse liquor showed a very low content of sulphur.

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Table 4.4: Elemental analysis of humic acids and lignin.

% elemental composition						
Extraction Method and material	C >C: high molecular w.t.	N >0.002%	C/N	S 0 – 2 %	(H + O)	Ash <1%
HApEuc	58.9	0.17	346.3	7.7	7.70	25.6
LHApEuc	58.7	0.20	293.7	4.3	31.68	5.1
HApAEuc	57.0	0.17	335.4	7.3	31.61	3.9
LHApAEuc	59.5	0.22	270.6	3.9	28.32	8.0
HApPin	50.9	0.16	318.4	6.2	15.53	26.2
LHApPin	60.5	0.22	274.9	1.9	30.49	6.9
HApAPin	47.9	0.17	281.8	5.9	37.19	8.8
LHApAPin	61.4	0.23	267.1	3.1	19.31	15.9
HApBag	55.8	0.85	65.7	0.3	37.53	5.5
HApABag	57.1	0.99	57.7	0.3	38.87	2.8
Na-HA	45.9	0.60	76.5	0.4	32.50	20.6
Leuc	55.6	0.13	427.5	4.5	22.39	17.4
Lpin	48.1	0.13	270.1	6.3	35.77	9.7

For definition of terms and acronyms see glossary.

Elemental analysis of S and Ash content of humic acids from black liquor

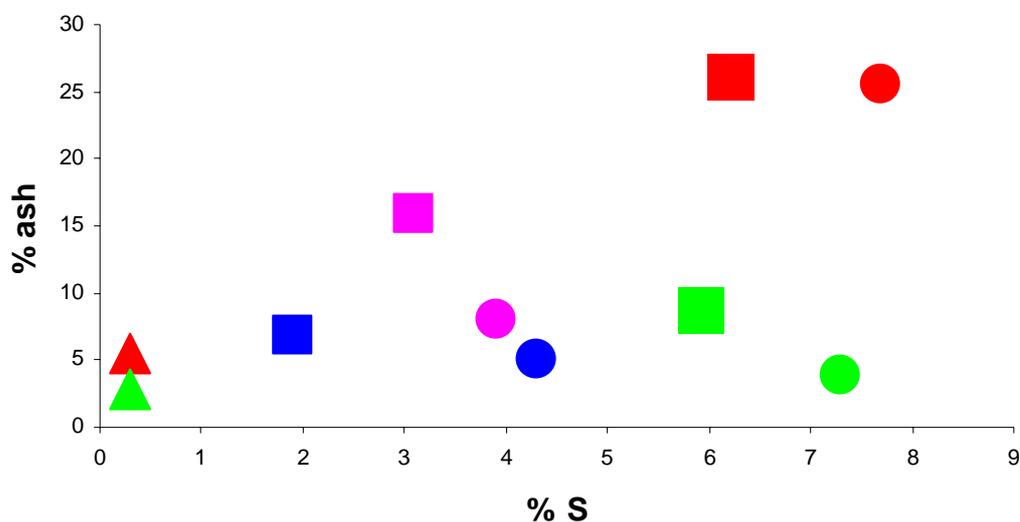


Figure 4.2: Variation of S and ash content with the method of extraction and black liquor material. **Red:** HAp, **Blue:** LHAp, **Green:** HApA, **Magenta:** LHApA  
**Circle:** HAEuc, **Square:** HAPin, **Triangle:** HABag.

The hydrogen and oxygen could not be analyzed with the available methods therefore; the values of hydrogen and oxygen (H + O) were presented as the percentage difference between

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C, N, S and ash content. In general, humic acids extracted from pine liquor exhibited higher (H + O) content than humic acids extracted from *Eucalyptus* liquor. Lignin extracted from pine liquor exhibited a higher (H + O) content than lignin extracted from *Eucalyptus* liquor. The ash content of humic acid varied with the method of extraction, however, it was noted that lignin extracted from *Eucalyptus* liquor exhibited higher ash content than lignin extracted from pine liquor. The E4/E6 ratios of humic acids increased in the following order: *Eucalyptus* liquor < Pine liquor < Bagasse liquor for the three different black liquor materials. However, the E4/E6 ratios of each black liquor material differed with the method of extraction (Table 4.2).

#### 4.5.2 Ultraviolet Visible (UV-VIS) Spectroscopy

Besides other materials as shown in Figure 4.3 the UV-VIS spectra of humic acids extracted from black liquor are shown. The spectra were similar to those of humic substances reported in literature showing a monotonical decrease in the spectra with increasing wavelength. Generally, all spectra showed a sharp peak in the region between 230 and 300 nm. A similar trend was observed between the lignin spectra and the spectra of humic acid both extracted from black liquor. The type of extraction method appeared to have an influence on the peaks that appeared at 250 and 350 nm.

#### 4.5.3 Fourier Transform Infrared (FTIR) Spectroscopy

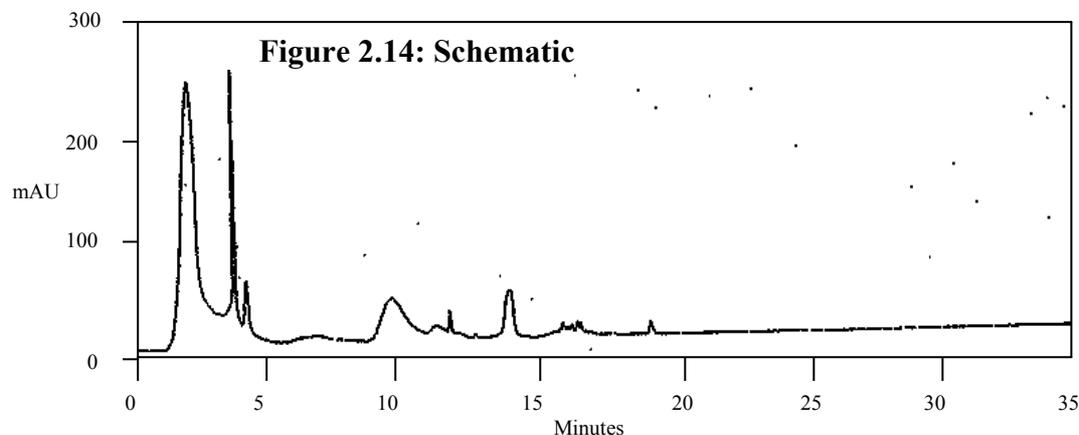
Besides other materials such as Na-HA, HA\_com and lignin, Figure 4.4 showed spectra of humic acids extracted from black liquor. The FTIR spectra of humic acids recorded some major bands which included, a broad band around  $3400\text{ cm}^{-1}$  (H-bonded OH groups) and a sharp peak around  $2900\text{ cm}^{-1}$  (aliphatic C-H stretch). Other bands were unstable peaks at  $1600\text{ cm}^{-1}$  (aromatic C=C, C=O, and/or C=O of bonded conjugated ketones, quinone, C=O stretch of amide) and bands in the region of  $1000 - 1400\text{ cm}^{-1}$ . Overall observations indicated similarities in the spectra of humic acids extracted from black liquor and Na-HA (Figure 4.4).

#### 4.5.4 High Performance Liquid Chromatography (HPLC)

Regarding high performance liquid chromatography the influence of pore size on the chromatographic separation is demonstrated in Figure 4.5. Dead time ( $t_0$ ) for the column was close to 5 minutes. By isocratic separation, using a mobile phase of acetonitrile : water using a stepwise gradient program and varying flow rate, most of the molecules of Na-HA were excluded. As observed in Figure 4.5 the exclusion peak was broader and clearly structured.

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This attributes to the partial penetration of the humic molecules into the pores of the stationary phase. The chromatograms of humic acids extracted from black liquor as well as those of Na-HA, HA\_com, and lignin are presented in Figures 4.6 (A to B).



**Figure 4.5: HPLC separations of Na-HA using neuclosile 1000-7 C18 column.**

It appears that all materials were successfully separated by the program of acetonitrile and water. HPLC chromatograms of humic materials indicated a resolution of a multiple of fractions in each case. The retention times ( $t_R$ ) for the first peak of each material is shown in Table 4.4.

Table 4.4: Retention times of humic materials.

Material	Retention time ( $t_R$ ) (minutes)
Humic acids	2
Lignin	0.5

Chromatograms for humic acids showed major differences around the area of the first peak (Figure 4.6A and B). Lignin samples show minor differences in the region between 2 and 3.5 min (Figure 4.6A).

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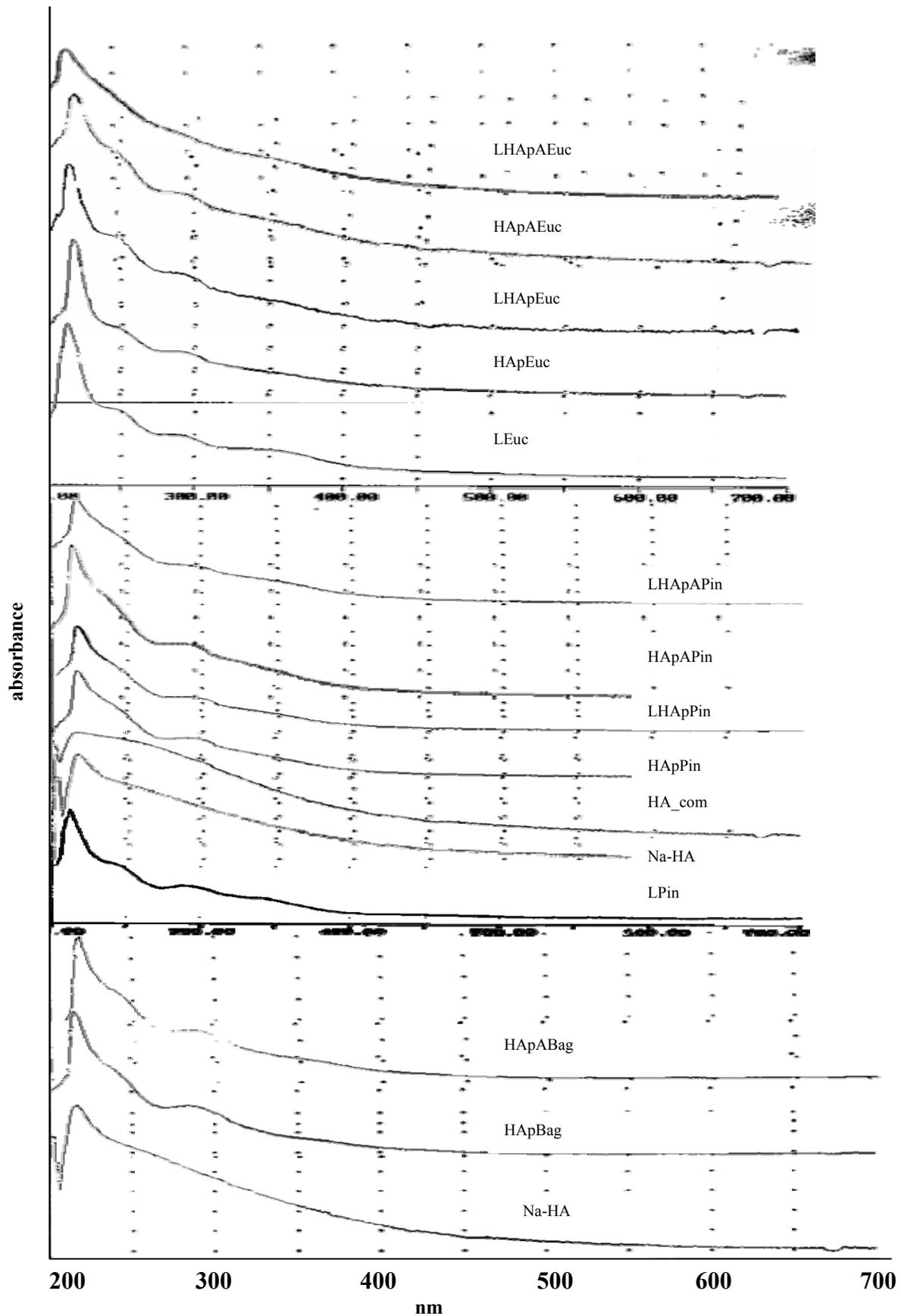
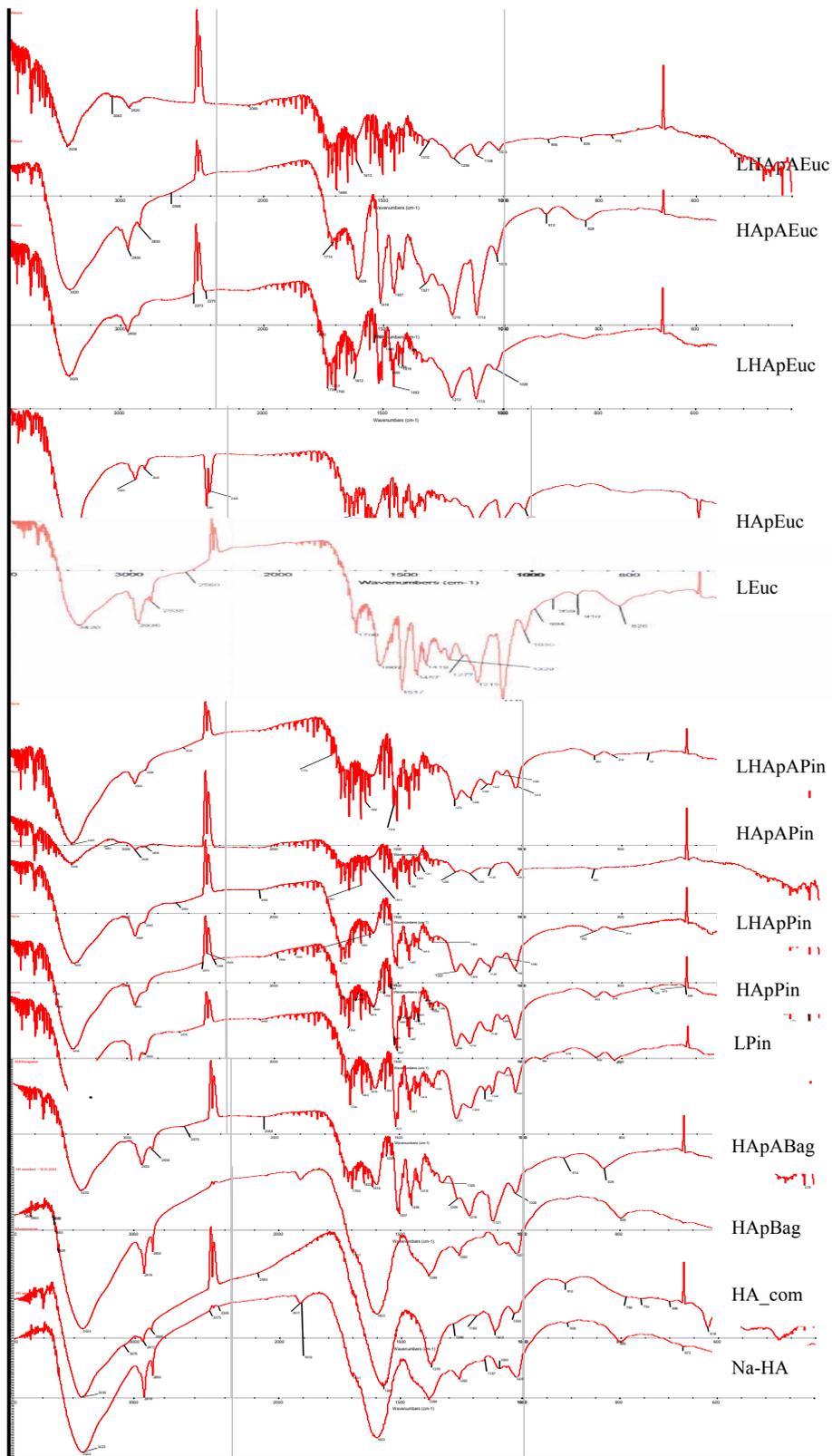


Figure 4.3: UV-VIS spectra of humic and lignin extracted from black liquor.

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**Figure 4.4: FTIR spectra of humic acids extracted from black liquor and other materials.**

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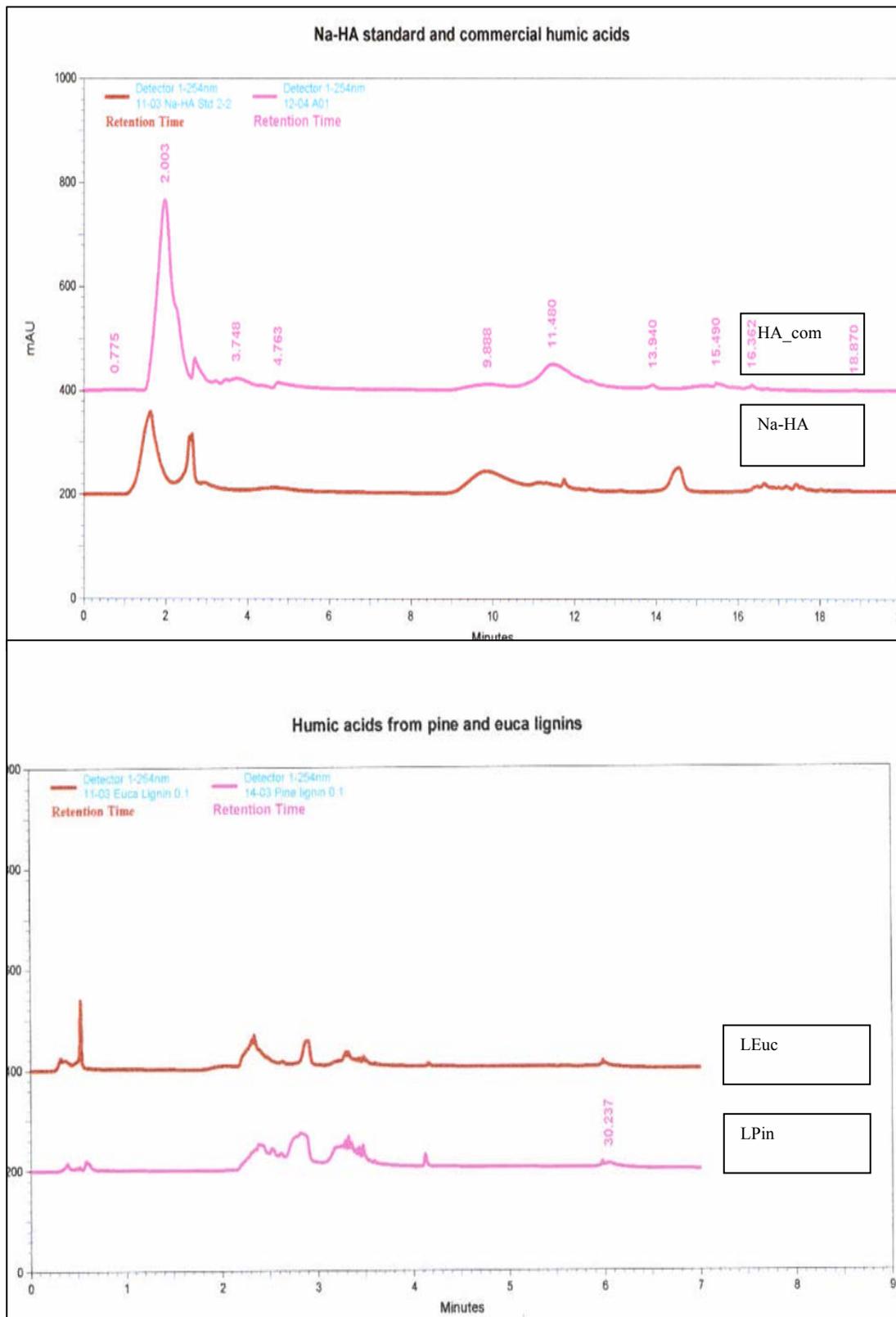


Figure 4.6: HPLC separations of Na-HA and lignin chromatograms.

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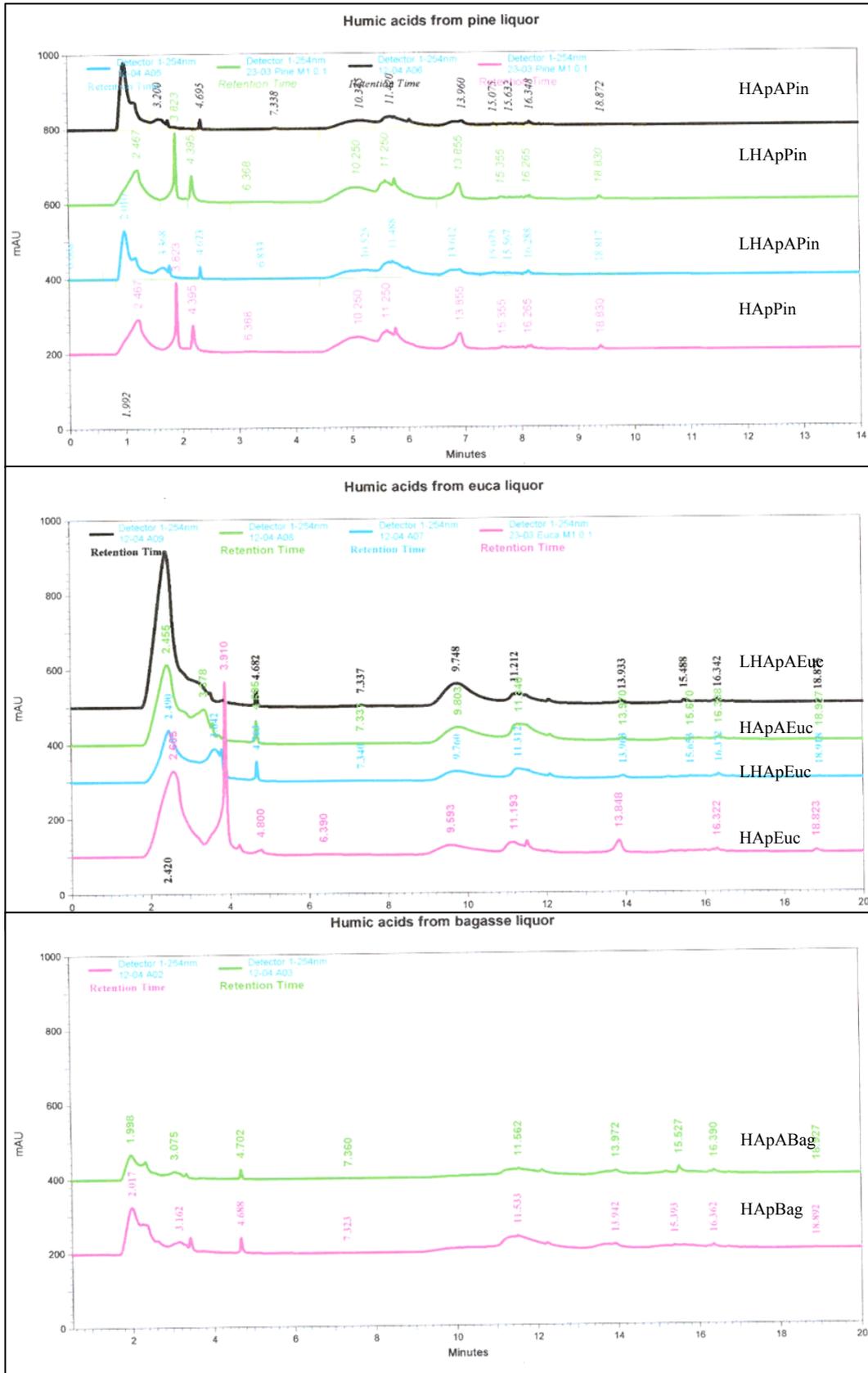


Figure 4.6B: Humic acid chromatograms

#### 4.6 DISCUSSION OF RESULTS FOR THE FORMULATION OF EXTRACTION METHOD AND QUALITATIVE ANALYSIS OF HUMIC ACIDS FROM BLACK LIQUOR

Extraction of humic acids from black liquor produced by Mondi Business Paper and Mondi Packaging Felixton using basic precipitation methods was possible. The four methods that were formulated namely: humic acid precipitation method (HAp), lignin extraction/humic acid precipitation method (LHAp), humic acid precipitation/acid treatment method (HApA) and lignin extraction/humic acid precipitation/acid treatment method (LHApA) were able to extract some humic acids from Kraft and bagasse black liquor. The highest yields of humic acids produced from both *Eucalyptus* and sugarcane bagasse were obtained by using the basic precipitation method (HAp) whilst for pine liquor this condition was observed whilst using HApA method. The quantity and quality of humic acids produced varied depending on the method of extraction employed (Table 4.3 and 4.4). HAp and HApA produced large quantities of humic acids whereas LHAp and LHApA produced small quantities of humic acids indicating that as a result of lignin pre-extraction the yields of humic acids from black liquor were negatively affected. The extraction of lignin from black liquor preceding the extraction of humic acids reduced the amount of humic substances in black liquor whilst on the other hand acid treatment washed away some fractions of humic substances from the humic acid sludge. To a large extent the production yields also depended on the type of material from which black liquor was produced i.e. *Eucalyptus* liquor, pine liquor or bagasse liquor. The sequence of production yields as affected by the method of extraction from each material is indicated as follows:

HAp: *Eucalyptus* > pine > Bagasse

LHAp: Pine > *Eucalyptus*

HApA: *Eucalyptus* > Pine > Bagasse

LHApA: Pine > *Eucalyptus*

For the above information see Table 4.3. None of the methods used for lignin extraction were appropriate for bagasse liquor as these methods were only applicable to black liquor produced by Kraft process. Generally, hardwood and softwood have lignin possessing different

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properties that respond differently to pulp production processes. In this study, hardwood showed a higher composition of lignin than softwood from the same amount of black liquor (*Eucalyptus*: 3.87% and Pine: 1.14 % from 1 l black liquor) (Table 4.3). This is in contrast with what is reported in literature where it is reported that conifer wood Lignins essentially made of G units are less susceptible to Kraft delignification than deciduous wood Lignins comprising both G and S units (Chiang et al., 1988). This implies that softwoods lignin is supposed to be sustained better than hardwood lignin during Kraft processing.

Chemical composition of humic acids was determined using elemental analysis to evaluate the suitability of humic acids for their application as fertilizer. The C, N, S and ash contents of humic acids are critical factors for determining functionality and nutrient value (Hunt et al., 2000; Abbt-Braun et al., 2004). Humic acids extracted from Kraft black liquors had substantially higher C, S, and ash contents, but low N content compared to humic acid standard (Table 4.4). Figure 4.2 shows that for the HAp method the ash content of HA from BLPin is 2 % above the ash content of HA from BLEuc and the ash content of HA from BLBag is 76 % lower than the ash content of HA from BLPin. For the HApA method the content of HA from BLEuc is 56 % lower than the ash content of HA from BLPin and the ash content of HA from BLBag is 68 % below the ash content of HA from BLPin. Therefore, for both HAp and HApA methods the ash content of HA from BLBag is below the ash content of HA from both BLEuc and BLPin. This variation is also observed in the ash contents of HA extracted using LHAp and LHApA methods but with different variation ranges.

The high sulphur as of humic acids extracted from black liquor results in a lower quality product for agricultural application. Though sulphur is an essential plant nutrient it could become an environmental pollutant if present in acidic forms and in excess (Zhao et al., 2006). The high sulphur content of humic acids extracted by HAp and HApA methods (Table 4.4) will therefore hinder the direct use of these products in agriculture limiting the use of these methods in the production of humic acids from black liquor. The ash content of humic acids extracted by the formulated methods in the present study was above the maximum acceptable value of 1 % (Ahmed, 2005). However, Na-HA also demonstrated high ash content (20.6%). Ash content reflects the degree of mineralization of humic acids and could be linked to the mineral content of the original raw materials and salts resulting from reactions during the pulping process. The reduction in ash content of humic acids extracted by LHAp method indicated the association of ash with lignin fractions in black liquor. The ash content of humic

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acid extract was also reduced by the HApA method, indicating that the phenolic or carboxylic compounds in black liquor may have reacted with the dilute acid treatment. Both the LHAp and HApA methods may therefore be used to increase the reactivity of the extracted humic acids by lowering the ash content. Low N content of these products resulted in high C/N ratios indicative of high molecular size. Though very low, the nitrogen content was quite constant regardless of the method of extraction. Compared to humic acids obtained from *Eucalyptus* and pine liquors humic acids obtained from bagasse liquor exhibited low C/N ratios. This was due to higher nitrogen content and low carbon content of humic acids obtained from bagasse liquor.

There were trends in humic acids obtained from each black liquor material that the carbon content varied with the type of extraction method employed in the extraction of humic acids. However, the carbon content of humic acids extracted from *Eucalyptus* liquor was quite constant irrespective of the method of extraction, being between 57.0 – 59.5% C but varied with the method of extraction for both humic acids extracted from pine and bagasse liquors. The carbon content of humic acids extracted from pine and bagasse liquors increased with the extraction of lignin and acid treatment processes indicating a high association of carbon materials with humic acids. Lignins extracted from *Eucalyptus* and pine liquor had carbon contents that were relatively lower than that of humic acids extracted from the same materials. An inverse trend was observed between humic acids that an increase in C/N ratio of humic acids was directly proportional to a decrease in sulphur content of humic acids. With lignin extraction and acid treatment the most important change in humic acids was a decrease in the C/N ratio. The quality of humic acids as determined by elemental composition influences the choice of the method of extraction for the large-scale production of humic acids hence the possible process flow. A method that produces humic acids with lower S, and ash contents will be the preferred method for the possible process flow for the production of humic acids at a large scale. According the result shown in Table 4.5 it appears that LHAp method is the preferred method over other methods especially with reference to pine liquor.

#### 4.7 INSTRUMENTAL ANALYSIS OF HUMIC ACIDS

Interpretation of spectra and HPLC of humic acids was based on literature (Duarte et al, 2003; Senesi and Miamo, 1994). General comparison of spectra and HPLC pointed out similar features regardless of the original nature of humic acid and the method of extraction. Humic acids extracted from black liquor exhibited spectroscopic features and molecular group

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components that were similar to those found in other humic substances reported in literature (see Chapter 2, Sec. 2.8). This indicated a fair comparison of the quality of humic acids extracted from black liquor to those found in other humic substances. The molecular structure of humic acids as determined by UV-VIS, FTIR and HPLC was mostly dependant on the source of the black liquor material rather than the method of extraction.

As observed by Björkman and Person (1957), Sarkanen et al. (1967a,b,c), Norrstrom (1972), Norrstrom and Teder (1971, 1973), and Lin and Detroit (1981), there were some minor differences observed in the ultraviolet spectra of humic acids especially in comparison to lignin spectra. These differences were suggestive of structural differences. The E4/E6 ratios (determined by UV-VIS spec.) of Lignins extracted from *Eucalyptus* (hardwood) liquor (E4/E6 = 3.71) and pine (softwood) liquor (E4/E6 = 7.27) showed that humic substances present in pine liquor were more degraded/humified than those present in *Eucalyptus* liquor. E4/E6 ratios showed that during the humic acid extraction processes humic substances found in pine liquor were recondensed (E4/E6 humic acids < E4/E6 lignin) whilst those present in *Eucalyptus* liquor were indicated to be more hydrolyzed (E4/E6 humic acids > E4/E6 lignin). This implies that during pulp production in a Kraft mill, lignin from *Eucalyptus* liquor is highly hydrolysed, thus changing its structure and properties. This is in agreement with the findings of Chiang et al. (1988) where they reported that hardwoods comprise of G and S units which make them susceptible to decomposition making humic substances in hardwoods more available.

For pine liquor it appeared that during extraction of humic acid, lignin fraction present in black liquor were condensed however, the extent of condensation depended on the method of extraction employed (E4/E6 ratios, Table 4.3). The standard method i.e. HAp method resulted in higher condensation (37 % condensation) of lignin fractions compared to 19 %, 18 % and 33 % condensation for LHAp, HApA and LHApA methods consecutively. The higher condensation of lignin fractions observed for HAp method is due to high content of lignin fraction in BL during the extraction of humic acids using this method. Acid treatment and lignin extraction results in humic acids of lower E4/E6 ratios as a result of pre-extraction of lignin fraction and degradation due to acid treatment. The humic acids standard i.e. Na-HA appeared to be highly aromatic compared to humic acids produced from *Eucalyptus* liquor, pine liquor and bagasse liquor as the E4/E6 ratio of this material was 3.2 with a standard deviation of 0.15 (Table 4.3). According to the E4/E6 ratios bagasse liquor was composed of humic substances that were highly hydrolysed owing to the volatility of non wood lignin

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material compared to wood material. Humic acid extracted from bagasse liquor showed very high E4/E6 ratios indicating a very low degree of condensation or low molecular size. The E4/E6 ratio of HA<sub>com</sub> was similar to the E4/E6 ratios of humic acids extracted from *Eucalyptus* liquor for all the extraction method except for HApA method (see Table 4.3). For *Eucalyptus* liquor HApA method appeared to promote condensation of lignin materials into aromatic compounds. For pine liquor all the E4/E6 ratios of humic acids were found within the range 3.5 – 5.9 reported by Kononova (1966). The spectra of humic acids particularly those derived from the pine and *Eucalyptus* liquor showed a decrease in maximum values of some peaks present in the lignin spectra. Depending on the type of extraction method the maxima at 280 and 350 nm present in lignin spectra appeared to flatten out in the spectra of humic acids suggesting the degradation in humic acids. This attribute was more evident in the spectra of humic acids extracted from *Eucalyptus* liquor (Figures 4.3).

Humic acid extracted from the pine liquor showed very minor changes from pine lignin as is the case in FTIR spectra. This suggested similarities in the structure of humic acids and lignin extracted from pine liquor. Humic acid from bagasse liquor showed a visible band at 280 nm. This was interpreted as an indication for the presence of high molecular weight structures possibly polysaccharides as there was no aromaticity confirmed by the E4/E6 ratios in the case of humic acids extracted from bagasse liquor. The method of extraction also seemed to have a notable influence on the structural appearance of the UV-VIS spectral peaks. For both humic acids extracted from pine and *Eucalyptus* liquor humic acids extracted by lignin extraction/humic acid precipitation/acid treatment (LHApA) method appeared to flatten out therefore losing the peaks at 250 and 280 nm than humic acids extracted using other methods. UV-VIS spectra of humic acids and lignin showed a maximum at 280 nm arising from O-substituted aromatic rings. A general trend was observed in the spectra of humic acids that as wavelengths increased, the spectra of humic acids decreased as recorded for many humic acids (Flaig et al, 1975; Schnitzer, 1990) while in lignin a relative maxima appeared in the 350 nm region due to conjugated double bonds with an aromatic ring in the side chains of lignin units. The spectra of humic acids extracted from bagasse liquor showed different features to that of Na-HA but similar features to those of humic acids extracted from *Eucalyptus* liquor and pine liquor. This may indicate that even though humic acids from bagasse liquor and Na-HA may have originated from the same material, humic acids extracted from bagasse may not have had enough time to mature as Na-HA (Figure 4.2).

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Humic acids extracted from bagasse liquor demonstrated clearly distinct FTIR peaks whilst the FTIR spectra of humic acids extracted from *Eucalyptus* and pine liquors exhibited many additional peaks (Figure 4.4). Comparatively, FTIR spectra for humic acids showed absorption peaks at  $3400\text{ cm}^{-1}$ , the sharp peaks around  $2900\text{ cm}^{-1}$ , the peaks at  $1600\text{ cm}^{-1}$ , and bands in the region  $1000\text{ cm}^{-1}$  which were assignable to the presence of  $-\text{OH}$  groups, aliphatic C-H stretch assuming the presence of  $-\text{OCH}_3$  groups, presence of aromatic structures, conjugated ketones, quinones, and stretch of amide and aromatic C=C and C=O stretching. The  $-\text{OCH}_3$  group was indicative of the lignin origin of humic acids extracted from black liquor. This attribute was confirmed by the reduction of the band in the region of  $2900\text{ cm}^{-1}$  in the spectra of lignin which occurred in the spectra of humic acids extracted from both *Eucalyptus* and pine liquor materials. The lignin spectrum of pine liquor indicated features suggesting close similarities between lignin and humic acids except for the observed reduction of the peak found in the region of  $2900\text{ cm}^{-1}$  in the spectra of humic acids (Figure 4.4). The spectra of humic acids extracted from *Eucalyptus* liquor do not show clearly distinct peaks in the region of  $1420 - 1700\text{ cm}^{-1}$  as shown in the spectrum of lignin. This behaviour is attributed to the higher content of lignin-derived aromatic structures compared to the structure of humic acids. The spectra of lignin extracted from *Eucalyptus* liquor also indicated bands at  $1215\text{ cm}^{-1}$ ,  $1115\text{ cm}^{-1}$  that showed guaiacyl (G) and syringyl (S) breathings typical in the spectra of lignin confirming what is reported in literature about the presence of G and S units in the lignin structure of *Eucalyptus* lignin.

Spectral attributes of humic acid extracted by humic acid precipitation/acid treatment method (HApA) from *Eucalyptus* liquor showed that acid-treatment encouraged condensation of lignin fractions. This was made evident by the recovery of the peaks in the region  $1420 - 1700\text{ cm}^{-1}$  in the spectrum of humic acid extracted using this method. The humic acids extracted from pine liquor exhibited peaks at  $\sim 1209$  and  $1123\text{ cm}^{-1}$ , thus showing an increase in intensity with decreased aromaticity. This feature is also confirmed by the high E4/E6 ratios of both lignin and humic acids extracted from pine liquor. These findings were similar to those obtained by Duarte et al. (2003). The similarity of the spectra of humic acids extracted from bagasse liquor with the spectrum of Na-HA confirmed the same originality as indicated in materials and methods. Overall observation of FTIR spectra showed that HApA method is cannot be recommended for the extraction of humic acids.

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The chromatograms of Na-HA, lignin and humic acids extracted from different black liquor materials are presented in Figure 4.5. At least five fractions were shown in all chromatograms regardless of the origin and method of extraction of humic acids as well as lignin. This symbolized the state of decomposition of lignin materials present in black liquor. The first peak at  $t_R$  (retention time) between 1.9 – 4.6 minutes corresponded to the excluded fraction. This was the area where major differences between the chromatograms were observed. Separation of the retarded fractions was mainly based on differences in hydrophobicity. Thus, humic acids extracted from *Eucalyptus* liquor, pine liquor and sugarcane bagasse liquor comprised of the largest contributions of hydrophobic features at  $t_R = 9.8$  min, 10.3 min and 11.8 min respectively. Observation of chromatograms for lignin molecules indicated that lignin fractions were highly excluded as they immersed before dead time. The fractions between 13 and 16.5 min became increasingly hydrophobic. Humic acids extracted from black liquor exhibited multiple peaks regardless of the method of extraction as did Na-HA. The main differences were found in the pattern of the peaks. All humic acids produced by the same method from the same black liquor material had the dominating peak always located at the same retention time. It appeared that the type of extraction method used to extract humic acids did not have much influence on the fractions of humic acids except on the peak intensity which was reduced after lignin extraction.

**4.8 SUMMARY**

Both quantitative and qualitative analysis indicates that black liquor produced during pulping process contains a substantial amount of humic acids materials that to a large extent resemble those found in nature. However, both yield and quality of these materials is directly influenced by the type of treatment to which black liquor is exposed to recover humic acids.

On the other hand, with the use of various analytical methods valuable information about humic acids was able to be recovered. Combination of various analytical techniques and instrumental analysis enabled the recovery of substantial information about the nature and structure of these materials.

## **CHAPTER 5. RESULTS AND DISCUSSIONS OF PHASE III AND IV**

### **5.1 INTRODUCTION**

This section discusses the results as obtained in the plant trial and a possible flow diagram for the extraction of humic acids from black liquor. The plant trial was conducted for humic acid products produced by the two most efficient methods i.e. HAp and HApA methods as shown in the yields and not on quality.

### **5.2 ASSESSING EFFECT OF HUMIC ACIDS ON PLANT GROWTH**

Results on the experiment conducted to assess the effect of humic acids produced by humic acid precipitation (HAp) and humic acid precipitation/acid treatment (HApA) methods on the growth-rate of the mustard plant are presented in this section. Figure 5.1 shows a laboratory setup for the plant trial.



**Figure 5.1: A laboratory scale of plant trials setup to test the effect of humic acids on plant growth.**

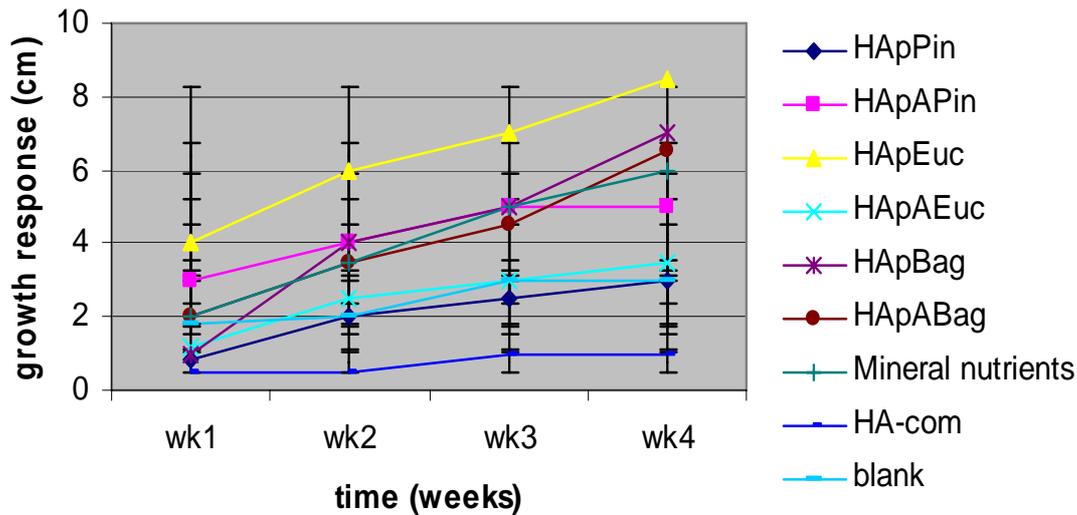
From Figure 5.1 it was observed that not all plants responded positively in terms of growth-rate to the application of humic acid and mineral nutrient materials. Plant response varied according to the fertilizer material applied

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Table 5.1: Response growth rate of mustard plant to the application of humic acids.

Growth period (4 Weeks)					
Growth rate (cm)					
Method extraction and material	Wk1	Wk2	Wk3	Wk4	Mean $\pm$ Stdev.
HApPin	0.8	2	2.5	3	2.1 $\pm$ 0.94
HApAPin	3	4	5	5	4.3 $\pm$ 0.96
HApEuc	4	6	7	8.5	6.4 $\pm$ 1.89
HApAEuc	1.2	2.5	3	3.5	2.6 $\pm$ 0.99
HApBag	1	4	5	7	4.3 $\pm$ 2.50
HApABag	2	3.5	4.5	6.5	4.1 $\pm$ 1.89
mineral nutrients	2	3.5	5	6	4.1 $\pm$ 1.75
HA_com	0.5	0.5	1	1	0.8 $\pm$ 0.29
Blank	1.8	2	3	3	2.5 $\pm$ 0.64

Controls

Effect of HA on plant growth (mustard: *brassica nigra*)

**Figure 5.2: Growth rate of mustard plant (*Brassica nigra*) in response to the application of humic acids as fertilizer.**

Table 5.1 and Figure 5.2 show the growth-rate response of plants (mustard seed) to humic acids and nutrient materials conducted over a period of 4 weeks. Generally, all peaks peaked on the ascending limb of growth rate. The growth rate of mustard plant in response to *Eucalyptus* liquor produced by the humic acids precipitation (HAp) method was generally highest on the ascending limb whilst that of commercial humic acid (HA\_com) was lowest.

### 5.3 DISCUSSION OF RESULTS ON THE EFFECT OF HUMIC ACIDS ON PLANT GROWTH

Depending on the type of extraction method (i.e. HAp or HApA) and black liquor material from which humic acids were extracted, humic acids showed an influence on plant (Mustard) growth. However, after a period of four weeks plant heights were relatively less than the general height of a mustard plant (*Brassica nigra*) which was indicated as 10 cm, Kirrhonffs, Margaret Roberts Seed Collection packet. This kind of plant response could possibly be attributed to the fact that the conditions under which this experiment was conducted were not ideal. There were some visible differences in the growth rate of plants based on the type of humic acid applied and extraction method used to extract the humic acids. The results shown in Table 5.1 and Figure 5.2 indicate that there was a slight difference in the height of the plants due to the type and method of the humic acid extraction. In certain cases a decline in the growth rate was observed which was probably due to depletion of humic acids in the soil medium or that the conditions were not quite favourable for the growth of the plant in question.

Data presented in Figure 5.2 is particularly important. It shows the connection between the humic acid solution and the response in growth-rate of mustard plants. Commercial humic acid (HA\_com), blank, and micronutrients were used as control group while humic acids extracted from black liquor were used as the treatment. Amongst the control materials HA\_com indicated a very poor effect on plant growth. As shown in Table 5.1 on average HA\_com indicated 0.8 cm as the maximum height that the mustard plant could reach in four weeks relative to the blank which gave the height of 3 cm. This was followed by humic acid produced by HAp method from pine liquor which showed no effect at all in comparison to the blank. From this observation, use of HAp method for extracting humic acids from pine liquor was evidently not the best method to consider since these humic acids appeared to show no stimulatory effect on the plant growth. This could be due to a number of factors such as concentration of humic acid, chemical content of humic acids and so on. Though the humic acids extracted by HApA method from pine liquor appeared to show a much better stimulatory effect on the growth rate of the plant this effect is no better than the application of mineral nutrients alone. Therefore, use of HApA method for the extraction of humic acids from pine liquor cannot be recommended either as a better alternative to the application of nutrients alone until further studies are considered that will perhaps vindicate other benefits

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such as their binding capacity of heavy metals to alleviate ground water pollution. With this understanding we can safely conclude that neither HAp nor HApA is good enough to extract humic acids from pine liquor that will be effective enough on plant growth. Humic acids extracted from *Eucalyptus* liquor and bagasse liquor could instead be recommended for plant growth with regards to yields but not with regards to quality. Their long term use may result in pollution due to high S and ash content.

Between humic acids extracted from *Eucalyptus* liquor and bagasse liquor, HAp method appeared to be a suitable method for the extraction of humic acids. A statistical approach i.e. t-test (Montgomery, 2005), was used to determine the significance in means differences regarding the growth rate of plant in response to humic acids extracted by HAp and HApA methods for both materials was conducted (Appendix 8.5). From this analysis it was observed that there was no significant difference on the effect of HA extracted from BLEuc and BLBag using HAp and HApA methods on plant growth. However, due to the high sulphur and ash content of these products more scientific experiments might be necessary to monitor the chemical compositions of humic acids. The method that gave better yields and better quality (2.51 % yields, 1.9 % S and 6.9 % ash) was LHAp methods when used to extract humic acids from pine liquor (Table 4.3 and 4.5) though the performance of this product on plant growth was not impressive.

#### **5.4 POSSIBLE CONTINUOUS PROCESS FOR THE PRODUCTION OF HUMIC ACIDS AT A LARGE SCALE**

A possible continuous process for the production of humic acids was formulated. This process was formulated based on the LHAp method which was found to be the method able to produce humic acids of better quantity and quality especially from pine liquor. This method appeared to show potential for the production of humic acids at a large scale without significantly compromising the quantity and quality of the product from pine liquor and might possibly work for the blend of pine and *Eucalyptus* liquor.

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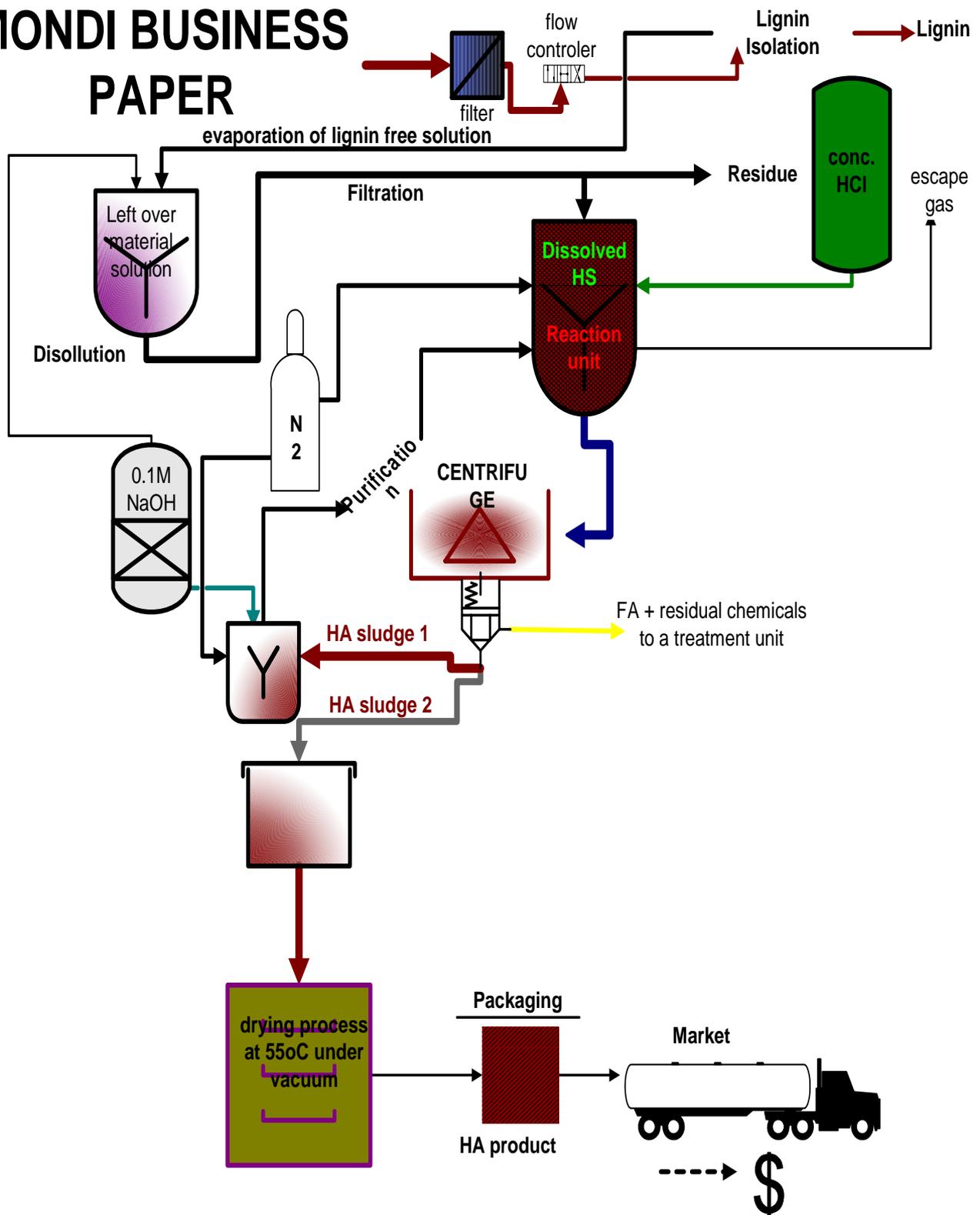


Figure 5.3: The possible process flow for the production of humic acids based on the LHAP method

### 5.4.1 Overview of the production process flows

In the production of humic acids, active chemicals consisted of hydrochloric acid (HCl) and sodium hydroxide (NaOH). The volume of chemicals and energy used in the process flow is not indicated for the reasons explained in Section 3.1. However, the change of these chemicals in this process can be expressed as the volume of chemical (with known concentration) per volume of black liquor produced depending on the pH and the nature of material (i.e. hardwood, softwood or bagasse material) from which black liquor is formed. Chemical reactions occur between the active dissolved organic and inorganic substances available in black liquor and effective HCl (actual amount of concentrated HCl). A large amount of HCl is consumed through acid base reaction. The presence of excess sodium hydroxide (NaOH) in black liquor keeps humic acid in solution because of the high pH ( $\text{pH} \geq 10$ ). However, addition of hydrochloric acid isolates an organic acid i.e. humic acid precipitate. The amount of HCl to be added is highly dependant on the pH of the solution which must be less than pH 2. At such low pH, all the humic acid present in black liquor should be able to precipitate. The production of humic acids in a batch process comprise of the following major components; reaction (i.e. precipitation by acidification of black liquor) vessel, nitrogen gas cylinder, centrifuge and vacuum oven. Each of these components, along with their equivalents in continuous production of humic acids will affect the quality and quantity of humic acid produced.

The time frame for the production of humic acids from black liquor can be investigated based on the type of extraction method. On the other hand, the amount produced in a determined space of time will depend both on the production method as well as availability of the raw material.

### 5.4.2 Availability of black liquor as raw material

Availability of black liquor depends on the production processes that take place in pulp and paper industry. Any changes in the production of pulp will have a significant impact on the amount of black liquor produced which in turn will have an impact on the production of humic acids.

The production of humic acids is outlined as a continuous process, utilizing black liquor produced by Mondi Business Paper (a Kraft process) and black liquor from Mondi Packaging Felixton (Bagasse black liquor). The basis of this process is that Mondi plant produces

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approximately 3100 tons of solids per day at the Richards Bay operation. However, despite the fact that black liquor from *Eucalyptus* wood (hardwood) and pine wood (softwood) were treated separately in the batch processes the volume of solids (i.e. 3100 tons) indicated is a combination of both hardwood and softwood materials suggesting that black liquor streams from both hardwood and softwood digesters are combined before they reach the evaporator. This calls for the investigation of the effect of humic acids produced from this blend on plant growth to see whether it's necessary to treat these materials separately or as combined. A conversation with Ciska Terblanche (Environmental Manager at Mondi Business Paper) made it clear that during pulp production the production plant utilizes more hardwood than softwood (Terblanche, 2005). The other important information to consider in the formulation of the production process flow is that flow rates of black liquor from hardwood range between 180-200 l/s on average whilst those of softwood vary up to 100 l/s (Appendix 8.4, Figure 8.4.2A) with the consistency of approximately 14% (Appendix 8.4, Figure 8.4.2B). This shows that to a large extent the flow rate is kept constant. Flow rates from Mondi Packaging Felixton could not be obtained due to confidentiality. Uncontrollable circumstances in pulp production processes timeously would cause the flow rates to drop consequently causing some imbalances in the plant. The design of the production plant for the production of humic acids would require that these conditions be taken into consideration. *Eucalyptus* and bagasse black liquor streams appear to produce satisfactory results on plant trials and since this is the final goal of this study ideally these streams would be recommended for the production of humic acids. However, the production of humic acids in this study looks at both quantity and quality of humic acids. Experiments showed that LHAp produces humic acids of better quantity (2.51 %) and quality (low S and ash content) from pine liquor compared to other methods and materials. Therefore, the possible process flow will be formulated based on this result i.e. LHAp not ignoring the fact that there are other procedures that can be adopted to improve the other methods.

The production process depicted in Figure 5.3 works as follows. Assuming that the production site of humic acids is located close to the source of black liquor as much as possible black liquor is directed through the piping system to humic acid production site. On its arrival at the site black liquor is filtered for the removal of suspended solids before it is processed for the production of humic acids. Preceding the production of humic acids is the extraction of lignin as defined in the batch process. This process lives behind a solution of black liquor assumed to contain no lignin but organic substances of lignin origin.

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Immediately after lignin pre-extraction the solution is filtrated and evaporated preferably spray-dried to remove the solvent. The powdered material is then dissolved in 0.1 M Na-OH and filtered to remove undessolved material. This solution is then directed through to the reaction tank where it is treated with HCl in the presence of nitrogen gas to precipitate humic acids whilst purging inorganic carbon like CO<sub>2</sub> and bicarbonates. The precipitation process is not selective to humic acids; therefore, the humic acid sludge may contain some impurities such as salts (e.g. NaCl) that can be removed through purification process such as ion exchange. The solution containing the sludge is then transferred to the centrifugation tank and centrifuged at 6000 rpm to recover the sludge (i.e. HA1) from the supernatant containing fulvic acids with chemical residuals (FA). The first humic acid sludge (HA1) is re-dissolved by adding 0.1 M NaOH to the sludge under nitrogen atmosphere. Before the sludge is re-precipitated, it is recommended that the humic acid solution is first purified to remove salts. In a batch process this can be achieved by dialysis method however, in a large-scale production process the use of a resin material (ion exchange resin) in a column chromatography can be considered in the event that no better and cheaper method is available. After the purification process, the humic acids solution is re-precipitated to coagulate humic acids relatively free of impurities.

The coagulated material is then centrifuged once to recover the humic acid sludge for the second time (HA2). The humic acid sludge is dried under vacuum conditions at 55°C to produce powdered humic acids. After the purification process humic acid in powdered to form the product is ready for the market.

## 5.5 DISCUSSIONS OF A POSSIBLE PROCESS FLOW

Lignin extraction/humic acid precipitation (LHAp) method was selected as the most suitable method for the production of humic acids at a large scale. However, it must be noted that the above illustration did not take into account the costs attached to the use of this method at a large-scale production of humic acids. In the formulation of the possible process flow it was assumed that the site for the large-scale production of humic acids will be located just close by the pulp production plant consequently cutting down the transport cost from the overall production cost. The outline of the process flow was made in such a way that there will be a continuous flow of black liquor from the pulp plant to the humic acid production plant. The change of chemicals such as HCl and NaOH used in this process can be expressed as the volume of chemical (with known concentration) per volume of black liquor produced

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depending on the pH and the nature of material (i.e. hardwood, softwood or bagasse material) from which black liquor is formed.

The analysis of humic acids accompanied by the plant trial tests showed that the use of *Eucalyptus* liquor and bagasse liquor as raw materials would yield better results than if pine liquor was to be used. However, their continual use would mean compromising the long term effects of high S and ash contents. Humic acids extracted from pine liquor using LHAp method were not evaluated on plant growth though they indicated a better quality compared to other humic acid products investigated in this study therefore, their stimulatory effect cannot be disregarded. A further investigation of humic acids extracted from pine liquor using LHAp method is recommended.

Depending on the amount of black liquor coming from the pulp industry use of multiple reactors may be recommended to control the flow-rate and the reactions thereof. To monitor the whole process a need to install a computer based system will be necessary to regulate pH during acid treatment, nitrogen gas and reaction pressure, transfer of sludge to the centrifuge, and separation of sludge from the effluents. Considering the fact that there might be a need to purify these substances a resin column may also be installed.

Determination of the mass transfer, energy consumption, and actual amount of input materials used could be done. This will assist in determining the actual cost involved in the production of humic acids using Lignin extraction/humic acid precipitation method. Generally, chemical analysis of humic acids from black liquor indicated high ash content. This may create challenges in application of these humic substances in a long-term agricultural use. More experiments would be necessary to determine the actual content of ash to avoid accumulation of heavy metals such as chromium, lead etc. that may inhibit plant growth in a long run.

## **CHAPTER 6. CONCLUSION AND RECOMMENDATIONS**

### **6.1 CONCLUSIONS**

There are various sources of humic substances from which considerable amounts of humic acids could be obtained. A comparative investigation of the hypothesis that black liquor is a possible source for the large-scale production of humic acids was established. The yield results showed that black liquor is more than 80 % better off in humic acid content compared to other humic substances that were investigated. As a result of this evaluation black liquor from pulp mills was chosen as an alternative sustainable source with potential for large scale production of humic acid especially that this is a readily available and sustainable material in South Africa than brown coal which is currently considered for large-scale production by most humic acid producers. Mining of brown coal for the production of humic acids may result in long effects such as erosion as this raw material may deplete from the land.

Identification of extraction method for the extraction of humic acids from black liquor was a success. Four extraction method namely, humic acid precipitation (HAp) method, Lignin extraction/humic acid precipitation (LHAp) method, Humic acid precipitation/acid treatment (HApA) method and finally Lignin extraction/humic acid precipitation/acid treatment (LHApA) method formulated were successfully used in the extraction of humic acids. However, each method showed a unique influence on the production yields and quality of humic acids as determined by analytical methods. The results from the experiments conducted clearly demonstrated that the quantity (yield), chemical composition, and structural complexity of humic acids obtained from black liquor vary depending on the type of black liquor material and the type extraction methods used.

HA extracted from BL Bag showed a very low ash and S content, however HA yields from this material were very low. This may be due to low lignin content of bagasse material. Irrespective of the method of extraction, HA from BLEuc gave high S and ash contents than HA extracted from BLPin. The high S content of HA extracted from BLEuc is associated with high lignin content (Table 4.3). This indicates that the S content of lignin from Eucalyptus wood material tends to be released into HA material during lignin degradation than the S content of lignin from pine wood material (Table 4.5). Application of acid wash in HApA

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method reduces the ash content of HA from BL. This shows that acid wash encourages dissociation reaction of macro elements thus replacing them with hydrogen atoms.

Lignin pre-extraction reduces the ash and S content relative to HAp method. Application of LHAp method shows that lignin is the primary source of both the ash and S content of humic acids extracted from BL. Application of acid wash after lignin extraction decreases the quality of HA. However, the effect of acid wash after lignin extraction on HA extracted differed with BL material (BLEuc and BLPin). For HA extracted from BLEuc acid wash reduced the S content while it increased the ash content but for HA extracted from BLPin the acid wash increased both S and Ash content. This is probably due to the response of lignin to chemical degradation influenced by the type of wood material. This in turn indicates different chemical composition of humic substances from these materials tending to respond differently when exposed to certain reactions. Application of LHAp method on BLPin produces good quality HA (low S and ash contents, Table 4.5) with acceptable yields (2.51 %) compared to BLEuc (1.55 %) (Table 4.3) making this a preferred method and BLPin a preferred material for the large-scale production of HA from BL. However, with more and improved techniques BLEuc may not be a bad source or a combination of both BLPin and BLEuc may require some investigation.

Although it was not possible to find a standard humic substance for use as a control, variations in the quantity and quality of humic acid in various materials assessed were apparent. It was possible to estimate quality of humic acids by determining the E4/E6 ratios and their effect on plant growth.

Humic acid produced from black liquor provides a potential source for organic fertilisers in South Africa's farming sector. The study demonstrated that the effect of humic acid on plant growth may be influenced by both the method of extraction and black liquor material. However, it also appears that not all humic acids may demonstrate such positive effects on plant growth. Some humic acids appeared to have no better effect on plant growth than the micronutrients used alone. This could be due to the conditions under which the experiments were conducted. For HAEuc and HABag produced using HAp and HApA methods the statistical tests demonstrated no significant difference on the effect of HA on plant growth.

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Although there are quite a number of processes leading to the production of humic acids such as humification by natural processes, it is evident from this study that chemical treatment of wood during pulp production actually shortens the humification process to just a few hours rather than waiting for days or even years. The type of wood material (i.e. softwood or hardwood) and bagasse material from which black liquor is produced may also affect the chemical and molecular composition of humic acids as shown in the variations of elements as determined in the elemental analysis.

Large-scale production of humic acids in South Africa is possible and would contribute tremendously towards reducing pollution to the environment by South Africa's pulp and paper industries. With proper process controls, and technological improvements in extraction methods, considerable amounts of humic acids of desired quality could be made available to the South African market at an economic rate. However, implications on the current alternative uses of black liquor, such as energy production, should be considered.

### 6.2 RECOMMENDATION

The following recommendations are suggested to improve the quality and to verify the effect of humic acids on plant growth:

- Determination of  $-OCH_3$  and  $-OH$  groups of humic acids and lignin produced from black liquor to investigate the effect of extraction method on the degradation of humic acids. This will also help determine whether there is a need to find ways that will lead to the degradation of lignin.
- Determination of the chemical composition of the ash content. Since there are metals that tend to inhibit plant growth such as lead, chromium etc. this analysis will help investigate whether humic acids extracted from black liquor do not contain such metals that may result in inhibitory effects. This will be of great importance especially for humic acids extracted from pine liquor as their effect on plant growth was not good. This analysis will help determine whether the behaviour of humic acids from pine liquor was due to the chemical composition of the ash content.
- Determination of sugar content of humic acid. When sugar reacts with nitrogen it has a tendency to produce pyridine which tends to be poisonous to plants. Since it appears that humic acids isolated from black liquor contain a low amount of nitrogen this calls for nitrogenation of these substances. If these humic substances must be nitrogenated

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then there is need to investigate their sugar content to avoid production of poisonous substances that may in turn inhibit plant growth.

- Determination of salt content. Accumulation of salt in the soils may lead to inhibitory effect on plant growth. By all means, humic acids with high salt content must be purified as their use may have long term effects on the soil and therefore on plant growth on which they are applied. Chromatographic purification (ion exchange) or dialysis may be ideal procedures to remove salt from humic acids in solution.
- Separation of the humic acid sludge from the solution during production of humic acids is a tedious exercise, a better method such as spray drying to do this separation rather than centrifugation may be considered. Centrifugation may not be user friendly at a large-scale hence a more spontaneous method that may require less labour is always a priority.
- With regards to assessment of humic acids on plant trials more assessments need to be conducted at a larger scale with all humic acid products extracted by the four extraction methods. This will help to confirm the findings of this study perhaps come up with more and better findings. A plant trial with a variety of plant materials needs to be conducted to reduce biasness.
- Determination of production costs as outline in the process outline needs to be investigated in order to establish the cost effectiveness of the method of extraction as recommended in this study.

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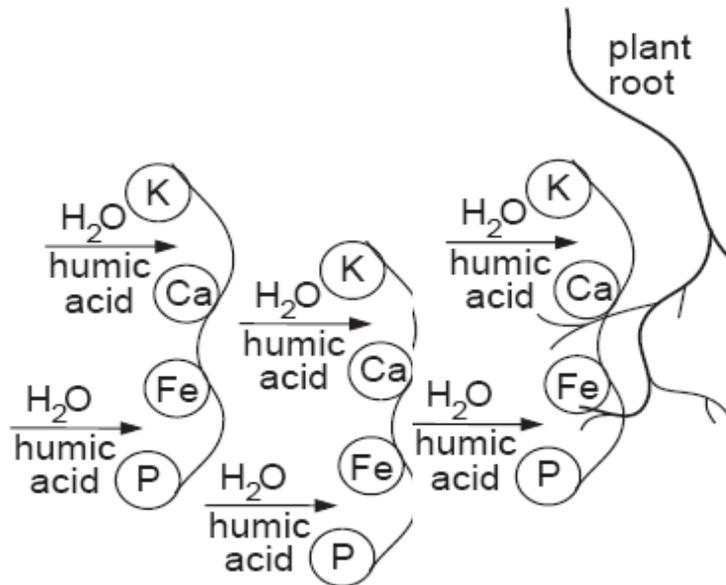
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APPENDICES

8. APPENDICES

Appendix 8.1



**Figure 8.1.1: Hypothetical micronutrients transfer by humic acids to the plant root system (Bio Ag Technologies International, 1999)**

Appendix 8.1.2

Yield calculation and comparison

1 ppm = ml/l

1g = 1 ml H<sub>2</sub>O

Therefore: 1 kg = 1000 ml H<sub>2</sub>O

Assumption: characteristics of GW are assumed to be the same as those of water (H<sub>2</sub>O).

**Yield C5W** = 0.222 g HA/50 ml solution of compost material  
= 0.004 g/ml

Therefore, % yield C5W = 0.004 \* 100 %  
= 0.4 %

**Yield GW** = 0.1042 g HA/50 ml sample  
= 2.084 g HA/l sample  
= 2.084 g HA/kg sample

Remember 1 ml H<sub>2</sub>O = 1 g (Density measured)

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$$1 \text{ l} = 1000 \text{ ml} = 1 \text{ kg}$$

$$\begin{aligned} \text{Therefore \% yield GW} &= 0.002084 \text{ kg HA/kg} * 100\% \\ &= 0.21 \% \end{aligned}$$

**Yield BL**

10 ml of conc. BL was weighed and found to be 12.4 g by weight.

$$10 \text{ ml BL} : 12.4 \text{ g} \approx 0.0124 \text{ kg}$$

$$x \text{ ml BL} : 1 \text{ kg}$$

$$\begin{aligned} x \text{ ml BL} &= 1 \text{ kg}/0.0124 \text{ kg} * 10 \text{ ml} \\ &= 806.45 \text{ ml} \end{aligned}$$

Therefore

$$806.45 \text{ ml BL} = 1 \text{ kg H}_2\text{O}$$

Then

$$1.31608 \text{ g HA} : 10 \text{ ml BL}$$

$$\begin{aligned} 10 \text{ ml BL} &= 1.3108 \text{ HA}/12.4 \text{ g BL} * 100 \% \\ &= 10.6 \% \end{aligned}$$

**Appendix 8.2**

Nature of lignin

Table 8.2.1: Lignin linkage frequencies and their reactivities.

Linkage	Softwood	Hardwood	Kraft Pulping – Phenolic OH			
			Free		Etherified	
			OH <sup>-</sup>	HS <sup>-</sup>	OH <sup>-</sup>	HS <sup>-</sup>
β-O-4	50	60	No	Yes	Yes	No/Yes*
α-O-4	2-8	7	Yes	No	No	No
β-5	9-12	6	No	No	No	No
5-5	10-11	5	No	No	No	No
4-O-5	4	7	No	No	No	No
β-1	7	7	No	No	No	No
β-β	2	3	No	No	No	No

\* No if α hydroxyl, yes if α carbonyl

## APPENDICES

**Appendix 8.3**

Table 8.3.1: List of chemicals and materials used in this study.

<u>Chemical or material</u>	<u>Function</u>
Magnetic stove	Moving the stirrer bar and heating
Small bottle with a stopper	Reaction container
Nitrogen gas cylinder	Purging in organic carbons during reaction.
Vacuum oven	For drying at a given temperature under vacuum.
MSE centrifuge	To remove suspended solids from solution.
Distilled water	To prepare solutions and solvents.
Weighing balance	Weighing the required mass
Concentrated HCl	For precipitation of humic acids
0.1 M NaOH	Extraction and dissolution of humic acids.
Ultraviolet visible spectra	To measure E4/E6 ratios and aromaticity and degradation degree of humic substances.
pH meter	To measure the pH during reaction.
0.45 µm membrane filter papers	To filter micron size suspended solids from the solution.
5 l vessel	Reaction vessels.
Motor stirrer	For continuous stirring the reaction mixture.
3000 cm <sup>3</sup> Plant Pots	To grow plants.
Mustard seed ( <i>Brassica nigra</i> )	The type plant species used during the plant trial.
Sand (grade twelve)	The type of soil used to growth plants
Ammonium sulphate [(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> ]	Source nitrogen as a micronutrient.
Mono Potassium Phosphate [H <sub>2</sub> KO <sub>4</sub> P]	Source of Potassium and Phosphate micronutrients.
Buckner funnel	For filtration purpose.

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Table 8.3.2: Parts per million (ppm) of nutrient element in solution when specified fertilizer compound is dissolved in water (Fertilizing greenhouse crops, 2006).

Fertiliser	Fertilizer & Element %	Amount/Gal	N	P	K
Potassium Nitrate	13.75-0-44.5 (36.9K)	1g	36	97	
Potassium Sulfate	0-0-50 (41.5K, 17S)	1g			110
Muriate of Potash	0-0-60 (49.8K, 45Cl)	1g			131
Mono Potassium Phosphate	0-22.8-28.7	1g		53	75
K-Mag of Sul-Po-Mag	0-0-22 (18K, 11Mg, 22S)	1g			48
Ammonium Nitrate	33.5-0-0	1g	41		
Ammonium Sulfate	21-0-0 (24S)	1g	88		
Urea	46-0-0	1g	55		
Nitric Acid	70 % HNO <sub>3</sub> (15.5N)	1g	121		
Diammonium Phosphate	18-46-0 (20P)	1g	41	53	
Phosphoric Acid	75%H <sub>3</sub> PO <sub>4</sub> (0.363g.P/ml)	1ml	47	96	

Note: For practical purposes, 3.5 ounces of fertilizer dissolved in 100 gallons of water yields the same ppm of element in solution as those given for 1g./gallon. (28.35gms.=1 ounce)

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Table 8.3.3: Fertilizer sources for the greenhouse crop production.

Name & Formula of Material	N-P-K	Analysis Others
Ammonium Chloride NH <sub>4</sub> Cl	25-0-0	0
Ammonium Nitrate NH <sub>4</sub> NO <sub>3</sub>	33.5-0-	0
Ammonium Phosphate (di) (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	21-53-0	0
Ammonium Phosphate (mono) NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub>	11-48-0	1.4% Ca, 2.6% S
Ammonium Sulfate (NH <sub>3</sub> ) <sub>2</sub> SO <sub>3</sub>	20-0-0	24% S
Calcium Nitrate Ca(NO <sub>3</sub> ) <sub>2</sub>	15-0-0	37% S
Sodium Nitrate NaNO <sub>3</sub>	16-0-0	0
Urea CO(NH <sub>2</sub> ) <sub>2</sub>	45-0-0	0
Urea-Formaldehyde	38-0-0	0
Superphosphate CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub>	0-20-0	18% Ca
Treble Phosphate CaH <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub>	0-42-0	12% S
Phosphoric Acid H <sub>3</sub> PO <sub>4</sub>	0-52-0	12% Ca
KCl	0-0-62	0
Potassium Nitrate KNO <sub>3</sub>	13-0-44	0
Potassium Sulfate K <sub>2</sub> SO <sub>4</sub>	0-0-53	0
MagAmp-Magnesium	7-40-6	18% S

APPENDICES

Appendix 8.4



a.



b.



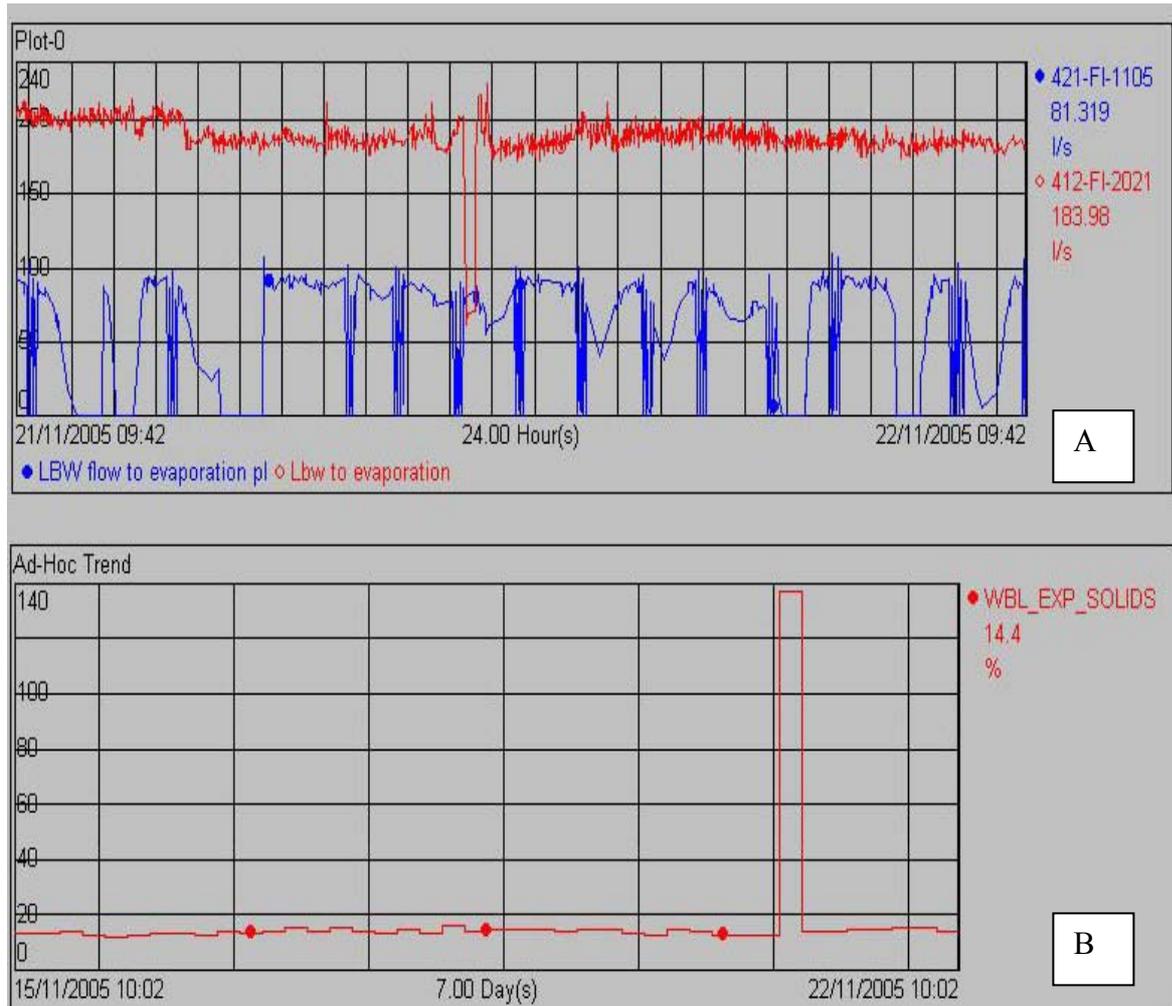
c.



d.

**Figure 8.4.1: Sample yields (a) powdered humic acid (b) powdered lignin (c) humic acids in solution (d) fulvic acid.**

APPENDICES



**Figure 8.4.2: Flow rates of black liquor at Mondi Business Paper and their consistency.**

- A: Red graph – flow rate of Eucalyptus black liquor  
 Blue graph – flow rate of pine black liquor
- B: consistency of flow rates.

## APPENDICES

## Appendix 8.5

Table 8.5.1: Determination of mean values and variance from the number of observations for both HAp and HApA method.

Time	HAEuc			HABag	
	HAp	HApA		HAp	HApA
Week1	4	1.2		1	2
Week2	6	2.5		4	3.5
Week3	7	3		5	4.5
Week4	8.5	3.5		7	6.5
$\hat{y}$	6.38	2.55		4.25	4.13
S	3.56	0.98		6.25	3.56
S <sup>2</sup>	12.69	0.95		39.06	12.69
n	4	4		4	4

HA humic acid

 $\hat{y}$  data mean

S variance

N number of observations

Calculations of t-distribution to determine the mean significance of humic acid precipitation method (HAp) and humic acid precipitation/acid treatment method (HApA)

$$t_0 = \frac{\hat{y}_1 - \hat{y}_2}{\sqrt{\frac{S_1^2 + S_2^2}{n_1 + n_2}}}$$

t-distribution for  
Bagasse liquor humic  
acids

$$\begin{aligned} t_0 &= \frac{6.375 - 2.55}{\sqrt{\frac{12.691 + 0.953}{4}}} \\ &= \frac{3.825}{\sqrt{\frac{13.644}{4}}} \\ &= \frac{3.825}{\sqrt{3.411}} \\ &= \frac{3.825}{1.847} \\ &= 2.071 \end{aligned}$$

$$\begin{aligned} t_0 &= \frac{4.25 - 4.125}{\sqrt{\frac{39.063 + 12.691}{4}}} \\ &= \frac{0.125}{\sqrt{\frac{26.372}{4}}} \\ &= \frac{0.125}{\sqrt{6.593}} \\ &= \frac{0.125}{2.568} \\ &= 0.049 \end{aligned}$$

$t_{0.25,6} = 2.97$  (Montgomery, 2005):  $t_0 > t_{0.25,6}$  in both cases.