Modification of lignin to produce soil conditioning materials

by

Qiniso Theo-Maxwell Ngiba

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Supervisor

Prof. Johann Görgens

Co-Supervisor/s

Dr. Luvuyo Tyhoda

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DECLARATION

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ABSTRACT

The decline in soil productivity demands intensive agricultural practices to maintain crop yields. Intensive agricultural practices such as tillage, low harvest residue retention, and the over-application of mineral nitrogen fertilizers lead to an unsustainable mineralization of soil organic matter (SOM). Increasing SOM may improve soil fertility for the long-term. Lignin is a precursor of humus – the stable form of SOM – and is available in abundance as a by-product from the pulp and paper industry. However, this raw material is devoid of plant nutrients and is characterized by high carbon-to-nitrogen ratios (C/N). Ammoxidation (treatment with oxygen and ammonia) incorporates nitrogen into lignin. This yields compounds with different nitrogen functional groups (organic and inorganic) that mineralize at different rates in the soil, giving the material a slow-release nitrogen characteristic.

In this study, kraft lignin (KRT), soda lignin (SOD) and sodium lignosulphonate (SLS) were characterized, ammoxidized, and assessed in a plant-growth trial. The unique properties of each raw material were investigated, and subsequently how these properties influenced nitrogen enrichment during ammoxidation. Kraft lignin contained more phenolic hydroxyl groups (6.05 mmol/g) compared to SOD (3.22 mmol/g) and SLS (0.37 mmol/g). FTIR revealed that SOD and KRT contained an appreciable quantity of methoxyl groups, compared to SLS. KRT was thus anticipated to incorporate more nitrogen compared to SOD and SLS due to its higher methoxyl and phenolic group contents. The oxidative degradation of these functional groups is essential to nitrogen incorporation.

Ammoxidation was conducted in a 1 L Parr reactor at 80 ^{o}C, 10 barg air pressure using 7 wt. % ammonia for 4 hours. The N-lignins had the following composition; N-KRT (2.8 N%, 18.3 C/N), N-SOD (1.9 N%, 28 C/N), and N-SLS (0.4 N%, 70 C/N). Pre-oxidation (1.5% H_2O_2) improved the nitrogen incorporation, yielding products with compositions: N-$O_{1.5}$-KRT (3.2 N%, 14.4 C/N), N-$O_{1.5}$-SOD (1.9 N%, 28 C/N), and N-$O_{1.5}$-SLS (0.5 N%, 48 C/N). However, only N-$O_{1.5}$-KRT met the criteria for N-lignins (C/N < 20). A stronger pre-oxidation (5% H_2O_2) yielded the following products; N-O_5-KRT (4.02 N%, 12.3 C/N), N-O_5-SOD (3.34 N%, 15.81 C/N), and N-O_5-SLS (1.61 N%, 14.64 C/N), which were suitable for use as soil organic amendments.

N-O_5-SLS had a salt index of 63.62%, which was notably higher than N-O_5-SOD (10.32%) and N-O_5-KRT (16.53%). This was attributed to its high ash content. The pH of this material (8.81 pH) was also beyond the desirable range of pH 5 - pH 7, in contrast to N-O_5-KRT (5.48 pH) and N-O_5-SOD (6.51 pH). The N-lignins increased the water retention capacity of sandy soil, making them attractive for use as soil amendments. Plants tests were conducted

over a 4-week period using mustard seedlings (*Sinapis alba*). Seedlings treated with N-O_5-SLS started yellowing after 3 days and withered within a week after the application of the material, attributed to its high pH (8.81 pH) and salt index (63.62%), corresponding to its high ash (61.14%) and sodium (144 900 mg/kg) contents. N-O_5-KRT (3.50 t/ha) and N-O_5-SOD (3.21 t/ha) yielded higher fresh mass than the control (2.04 t/ha).

It was concluded that KRT and SOD possessed desirable properties for ammoxidation: high phenolic hydroxyl group content, high methoxyl group content, and ease of purification (through acid precipitation) to make low ash raw materials, compared to SLS. Further studies may assess the profitability of the industrial production of N-lignins from local industrial lignins, conduct long term studies on the application of N-lignins as soil rehabilitation materials, and assess the suitability of these lignins for use in different soil types and plant species.

OPSOMMING

Die afname in grondproduktiwiteit vereis intensiewe landbougebruike om gewasopbrengste te handhaaf. Intensiewe landbougebruike soos grondbewerking, lae oesresiduretensie, en die oor-toepassing van minerale stikstofkunsmis lei tot ’n onstabiele mineralisering van grond organiese materiaal (SOM). Verhoogde SOM mag grondvrugbaarheid vir die lang-termyn verbeter. Lignien is ’n voorloper van humus – die stabiele vorm van SOM – en is beskikbaar in oorvloed as ’n byproduk van die pulp-en-papier-industrie. Hierdie roumateriaal is egter sonder plantnutriënte en is gekarakteriseer deur hoë koolstof-tot-stikstofverhoudings (C/N). Ammoksidasie (behandeling met suurstof en ammonia) inkorporeer stikstof in lignien. Hierdie lewer samestellings met verskillende stikstof funksionele groepe (organies en anorganies) wat mineraliseer by verskillende tempo’s in die grond, en gee die materiaal ’n stadig vrylaatbare stikstof karakteristiek.

In hierdie studie, is kraft lignien (KRT), soda lignien (SOD) en natrium lignosulfonaat (SLS) gekarakteriseer, ammoksideer, en geassesseer in ’n plantgroeitoets. Die unieke rou-materiaal is ondersoek, en vervolgens hoe hierdie eienskappe stikstofverryking gedurende ammoksidasie beïnvloed. Kraft het meer fenoliese hidroksielgroepe bevat (6.05 mmol/g) in vergelyking met SOD (3.22 mmol/g) en SLS (0.37 mmol/g). Fourier Transform Infrarooi (FTIR) het getoon dat SOD en KRT ’n merkbare hoeveelheid metoksielgroepe bevat het, in vergelyking met SLS. Dit was dus verwag dat KRT meer stikstof sou inkorporeer in vergelyking met SOD en SLS as gevolg van sy hoër metoksiel en fenoliese groep-inhoud. Die oksidatiewe degradasie van hierdie funksionele groepe is essensieel vir stikstof inkorporasie.

Ammoksidasie is uitgevoer in ’n 1 L Parr-reaktor by 80 °C, 10 bar lugdruk deur 7 wt.% ammonia vir 4 ure te gebruik. Die N-lignien het die volgende komposisie: N-KRT (2.8 N%, 18.3 C/N), N-SOD (1.9 N%, 28 C/N), en N-SLS (0.4 N%, 70 C/N). Voor-oksidasie (1.5% H_2O_2) het die inkorporasie van stikstof verbeter, en produkte met die volgende samestellings opgelewer: N-$O_{1.5}$-KRT (3.2 N%, 14.4 C/N), N-$O_{1.5}$-SOD (1.9 N%, 28 C/N), en N-$O_{1.5}$-SLS (0.5 N%, 48 C/N). Net N-$O_{1.5}$-KRT het egter die kriteria vir N-lignien ($C/N < 20$) bereik. ’n Sterker voor-oksidasie (5% H_2O_2) het die volgende produkte opgelewer: N-O_5-KRT (4.02 N%, 12.3 C/N), N-O_5-SOD (3.34 N%, 15.81 C/N), en N-O_5-SLS (1.61 N%, 14.64 C/N), wat gepas was vir gebruik as grond organiese wysigings.

N-O_5-SLS het ’n soutindeks van 63.62% gehad, wat aansienlik hoër was as N-O_5-SOD (10.32%) en N-O_5-KRT (16.53%). Dit is toegeskryf aan sy hoë asinhoud. Die pH van hierdie materiaal (8.81 pH) is ook buite die gewenste bestek van pH 5 tot pH 7, in kontras met N-

O_5-KRT (5.48 pH) en N-O_5-SOD (6.51 pH). Die N-ligniene het die waterretensiekapasiteit van sanderige grond verhoog, wat dit aanloklik maak vir gebruik as grondwysigings. Planttoetse is uitgevoer oor ’n 4-weke periode deur mostertsaailinge (*Sinapis alba*) te gebruik. Saailinge behandel met N-O_5-SLS het geel begin word na 3 dae en het verwelk binne ’n week na die toepassing van die materiaal, toegeskryf aan sy hoë pH (8.81 pH) en soutindeks (61.14%) en natriuminhoud (144 900 mg/kg). N-O_5-KRT (3.50 t/ha) en N-O_5-SOD (3.21 t/ha) het hoër vars massa as die kontrole (2.04 t/ha) gelewer.

Dit is vasgestel dat KRT en SOD begeerlike eienskappe vir ammoksidasie het: hoë fenoliese hidroksielgroepinhoud, en gemak van suiwering (deur suurpresipitasie) om lae asroumateriale te maak, in vergelyking met SLS. Verdere studies kan die winsgewendheid van die industriële produksie van N-lignien uit plaaslike industriële lignien assesseer, langtermynstudies op die toepassing van N-lignien as grondrehabiliteerdermateriale uitvoer, en die geskiktheid van hierdie ligniene vir die gebruik in verskillende tipes grond en plantspesies assesseer.

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Lord, so many times I fail; I fall into disgrace. But when I trust in you, I have a strong and glorious presence protecting and anointing me.

Forever you’re all I need!

Psalms 73:26

The Passion Translation (TPT)

“The thin layer of soil covering the earth’s surface represents the difference between survival and extinction for most terrestrial life”

Doran and Parkin, 1994

ABBREVIATIONS

AOAC	Association of Analytical Chemists
C_α	Carbon atom at the α-position of a phenyl propane unit
C_β	Carbon atom at the β-position of a phenyl propane unit
C_γ	Carbon atom at the γ-position of a phenyl propane unit
CEC	Cationic Exchange Capacity
EC	Electrical conductivity
FTIR	Fourier Transform Infrared Spectroscopy
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
ICP – OES	Inductively Coupled Plasma Optical Emission Spectrometry
HPLC	High Pressure Liquid Chromatography
KRT	Kraft lignin
LC	Liquid Chromatography
LCC	Lignin carbohydrate complexes
N-KRT	Ammoxidized Kraft lignin
N-SLS	Ammoxidized sodium lignosulphonate
N-SOD	Ammoxidized Soda lignin
N-$O_{1.5}$-KRT	Pre-oxidised (1.5%H_2O_2) and ammoxidized kraft lignin
N-O_5-KRT	Pre-oxidised (5%H_2O_2) and ammoxidized kraft lignin
N-$O_{1.5}$-SLS	Pre-oxidised (1.5%H_2O_2) ammoxidized sodium lignosulphonate
N-O_5-SLS	Pre-oxidised (5% H_2O_2) ammoxidized sodium lignosulphonate
N-$O_{1.5}$-SOD	Pre-oxidised (1.5% H_2O_2) ammoxidized soda lignin
N-O_5-SOD	Pre-oxidised (5% H_2O_2) ammoxidized soda lignin
NSSC	Neutral Sulphite Semi-Chemical pulping
NUE	Nitrogen Use Efficiency
NUA	Nitrogen uptake as a percentage of applied nitrogen
O-KRT	Pre-oxidised Kraft lignin
O-SLS	Pre-oxidised sodium lignosulphonate
O-SOD	Pre-oxidised Soda lignin

SI	Salt index
SLS	Sodium lignosulphonate
SOD	Soda lignin
Sob-N	Strong organically bound nitrogen
SOM	Soil Organic Matter
TAPPI	Technical Association of the Pulp and Paper Industry

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1 INTRODUCTION

1.1 Background

Mineral fertilizers have supported high crop yields in the past. However, there is now a yield plateau: yield increases per unit of fertilizer applied are declining and nutrient use efficiencies (NUE) are now lower than 50% (Johnson and Raun, 2003). This is due to the decline in the productivity of the soil, which demands intensive agricultural measures to maintain or increase crop yields to feed the growing global population (Basosi *et al.*, 2014; Rickson *et al.*, 2015; Sharma and Bali, 2017). Intensive agricultural practices such as tillage, low harvest residue retention, and the over-application of mineral nitrogen fertilizers lead to unsustainable SOM mineralization and high erosion rates. As a result, soil erosion in agricultural land occurs at a rate that is 10 to 40 times higher than the rate of natural soil formation (Pimentel and Burgess, 2013; Gomiero, 2016). SOM promotes soil aggregate formation, improves cationic exchange capacity (CEC), and supports the soil microbial community. Its rapid mineralization disturbs the soil structure, depletes soil nutrients, makes the soil prone to erosion, and limits biological activity, leading to a decline in soil productivity (Krull *et al.*, 2004; Crews and Rumsey, 2017).

It is thus imperative to identify sustainable methods to improve SOM in agricultural fields and to rehabilitate degraded soils. The application of organic residues such as sewage sludge, composts, peat, and manure, is the conventional method used for improving SOM (Rickson *et al*., 2015). However, organic residues may cause heavy metal pollution, and are often not available in the desired quantities in areas suffering from severe degradation (Ragab, 2008; Tyhoda, 2008; Zanella *et al*, 2018). Furthermore, organic residues typically have high carbon-to-nitrogen (C/N) ratios. Their sole application can result in a net immobilization of nitrogen in the soil (during their degradation), causing nitrogen deficiencies for crops (Khalil *et al.*, 2007; Luce *et al.*, 2011; Liu *et al.*, 2020). Soil organic amendments need to have C/N ratios of less than 20 to cause a net mineralization of nitrogen in the soil – making nitrogen available for plant uptake and increasing SOM content (Fischer and Schiene, 2002) .

Nitrogen supplied through the application of mineral nitrogen fertilizers is immediately available for plant uptake, due to the high solubility of these materials. However, this also makes nitrogen susceptible to various loss mechanisms such as ammonia volatilization, nitrate denitrification, and nitrate leaching. This leads to lower nitrogen use efficiencies (demanding higher fertilizer inputs), and harmful environmental and health effects (Basosi *et al.*, 2014; Khan *et al.*, 2017). The accumulation of nitrates in water streams causes eutrophication, while nitrogen oxides in the atmosphere contribute to global warming (Basosi *et al.*, 2014). A high concentration of nitrates (> 10 mg/ℓ) in drinking water is also a health hazard. It can result in a condition called methemoglobinemia – an elevated methemoglobin level in the blood. This reduces the oxygen-carrying capacity of the blood, especially in infants (Johnson, 2019).

There is a need to develop soil conditioning products that release nitrogen over an extended period and improve SOM. In this regard, nitrogen-releasing, soil-conditioning materials have been synthesized from lignin through a process termed oxidative ammonolysis or ammoxidation (the term ammoxidation will be used henceforth)(Fischer and Schiene, 2002). Products obtained from this process are referred to as N-lignins. In this process, lignin is treated with oxygen and ammonia. This binds nitrogen into lignin in different functional groups (mineral and organic). These functional groups mineralize at different rates in the soil, resulting in a slow-release characteristic (Meier *et al*., 1994; Fischer and Schiene, 2002; Tyhoda, 2008). This is an attractive option because lignin is a precursor of humic substances in the soil (Beckham, 2018; Pramudono *et al.*, 2018). Furthermore, the application of lignin in the soil has also been reported to increase erosion resistance and inhibit nitrification (Huang *et al.*, 2003; Zhang *et al.*, 2020).

Ammoxidation is analogous to natural humus formation. Both these processes involve oxidation, demethylation, demethoxylation, and the cleavage of aromatic structures (Fischer and Schiene, 2002). Various technical lignins have been used as raw materials for ammoxidation, yielding products with 3% to 5% nitrogen, and C/N ratios of less than 20 (Meier *et al.*, 1994; Ramírez *et al.*, 1997, 2007; Tyhoda, 2008). Nitrogen is made plant available through the degradation of the molecular structure of lignin by soil microorganisms, and through the chemical degradation of functional groups (e.g., hydrolysis) (Ramírez-Cano *et al.*, 2001).The aim of this study was to assess the suitability of industrial lignins from South African pulp and paper mills for use as raw materials in the synthesis of N-lignins.

1.2 Motivation for the study

The rapid mineralization of SOM leads to a decline in soil productivity and increases atmospheric carbon dioxide levels. South African soils generally have a low SOM content, as evidenced by low organic carbon contents. According to Du Preez *et al.* (2011), only 4% of South African soils contain more than 2% soil organic carbon (SOC), while 58% of soils contain less than 0.5% SOC, as shown in Table 1 below. The minimum SOC level for low fertility soil is 1.2% (Musinguzi *et al.*, 2016). Thus, 58% of soils in South Africa do not meet the criteria for minimum fertility.

Table 1: Percentage organic carbon in South African soils (Du Preez *et al.,*2011)

Soil organic carbon	Percentage of South African soils
Less than 0.5%	58%
0.5% to 2.0%	38%
More than 2.0%	4%

As a result, only ~12% of the available land area is suitable for agricultural production (Schulze *et al.*, 2020). Sustainable methods of improving the SOM content and rehabilitating degraded soils are required (Scotti *et al.*, 2015). Copious quantities of lignin are available as by-products in spent pulping liquors, especially from sulphite pulping mills (Boshoff, 2015). The production of N-lignins from these liquors can thus be a process route that;

- Valorises lignin by-products from pulp and paper mills, and reduces pressure on recovery boilers in these mills, and
- Produces soil conditioning materials to improve SOM

1.3 Structure and presentation of the thesis

Chapter 1: Introduces the study and provides a background understanding of the ammoxidation process. This chapter also elaborates on the need for considering South African lignins as raw materials and the problems that N-lignins could potentially address.

Chapter 2: This chapter provides a theoretical understanding of lignin; its molecular structure and how different pulping techniques modify it. It also expounds about soil degradation and provides the state of the art in lignin ammoxidation.

Chapter 3: Outlines the methodology undertaken for the characterization of both the starting materials and the products. Also reports on how the ammoxidation and pre-oxidation reactions were performed.

Chapter 4: Presents the results and discussion on the differences observed in each raw material and their performance during ammoxidation.

Chapter 5: Presents the methodology, results, and discussion of the pot trial assessment of the N-lignins

Chapter 6: Presents conclusions on the study and recommendations for further research.

2 LITERATURE REVIEW

2.1 Soil degradation

2.1.1 Definition and extent

Soil degradation refers to a deterioration of the soil productivity because of physical (erosion and compaction), chemical (acidification and salinization), and biological (loss of SOM and soil microorganisms) processes (Gomiero, 2016;Kopittke *et al.*, 2019). About 98.8% of the food consumed by human beings comes from the soil, while the difference is obtained from aquatic sources. The rapid increase in the global population from 6.1 billion in year 2000 to an estimated 9.8 billion by the year 2050, is placing a huge demand on soils (Smil, 1999; Rickson *et al.*, 2015). Since 1950, agricultural intensification (the increase in crop yield per unit area) has been the main route used to increase agricultural production, as opposed to expanding agricultural land. However, current agricultural intensification practices lead to an unsustainable soil degradation (Kopittke *et al.*, 2019).

Current farming practices significantly decrease the SOM content, making soil a net source of atmospheric carbon dioxide (Kopittke *et al.*, 2019). This decreases the soil's resistance to erosion, it's water holding capacity, and the overall soil productivity (Rickson *et al.*, 2015). This then demands more fertilizer inputs and irrigation, resulting in an inefficient use of fertilizer nutrients. The nitrogen NUE is reported to be less than 50% and averages 33% worldwide (Johnson and Raun, 2003). Sustainable agriculture aims to maintain soil fertility, increase SOM content and minimize soil erosion (Gomiero, 2016). However, Pimentel and Burgess (2013) reported that about 80% of global agricultural land is affected by mild to severe degradation. This section discusses soil degradation processes and how they are related to agricultural intensification practices.

2.1.2 Agricultural intensification and soil degradation

Mineralization of SOM

The disturbance of the soil during tillage increases SOM decomposition rates. This occurs through the destruction of soil aggregates and the structure of the soil, which then exposes more SOM to decomposition (Crews and Rumsey, 2017). Furthermore, many modern farming practices combine frequent tillage with low residue retention in the soil, resulting in a rapid decrease in the SOM content of soils with little replenishment (Rickson *et al*., 2015). The burning of harvest residues is a widespread practice in the cultivation of maize, rice, and other crops. This practice helps to control insects and reduces fieldwork for the next cropping period. However, it decreases the amount of organic inputs into the soil (Gomiero, 2016; Kopittke *et al.*, 2019). As a result, soil microbial communities are also destructed. When forests are cleared for agricultural use, perennial vegetation is removed and replaced by vegetation that lasts for a shorter period (Crews and Rumsey, 2017). Over time, the litter layer disappears resulting in a decrease in the organic residue input and a reduction in the number and diversity of soil microorganisms.

Soil erosion

Soil erosion is the removal of a thin layer of topsoil under the action of wind, water and other erosion agents. An estimated 75 billion tons of soil is eroded annually. Two thirds of this are from agricultural land (Pimentel and Burgess, 2013; Larney *et al.*, 2016). Agricultural land is more prone to erosion because of tillage and cultivation practices (Six *et al.*, 1998). The loss of 1 mm of soil accounts for a loss of 15 t/ha, yet it can easily go unnoticed (Pimentel and Burgess, 2013). Soil erosion selectively removes finer soil fractions that are crucial for crop growth. The soil that leaves with erosion agents (water, wind, and co-extraction on agricultural equipment) contains about three times more nutrients, and 1.3 to 5 times more SOM per unit mass compared to the soil that remains (Pimentel and Burgess, 2013; Gomiero, 2016; Kopittke *et al.*, 2019). Furthermore, soil erosion decreases the rooting depth for crops. Soil erosion occurs much more rapidly in agricultural land compared to land not used for cultivation , and exceeds the rate of natural soil formation (Pimentel and Burgess, 2013; Gomiero, 2016).

Soil compaction

Soil compaction occurs when soil is compressed by an applied force, originating from the weight of machinery or animals. This reduces the pore spaces between soil aggregates (Crews and Rumsey, 2017). Compaction results in the integration of soil particles and aggregates which increases shear strength (Rickson *et al.*, 2015). This limits water permeability and aeration, hinders seed germination on the surface of the soil, hinders root elongation, and limits the foraging range in subsoil. The factors that affect soil compaction are weight of vehicles, number of vehicle passes per unit area, the moisture content of the soil, and the number of animals grazing per unit area. These factors are expected to increase with agricultural intensification (Shah *et al.*, 2017). Soil compaction can reduce crop yields by 20% to 55% (Gomiero, 2016). Ploughing and subsoiling can reduce the effect of compaction. However, this is an expensive option. Preventative measures such as not driving or walking across the land are not practical (Gomiero, 2016; Kopittke *et al.*, 2019). Soil organic matter improves aggregate strength and water infiltration in the soil. Thus, it is important to improve the resilience of soils to compaction by increasing the SOM content of the soil (Rickson *et al.*, 2015).

Loss of soil biodiversity

Soil microorganisms are responsible for various chemical processes in the soil such as nitrogen cycling and organic matter decomposition. They also engage in symbiotic relationships with plants, which improves the nutrient use efficiency (Young *et al.*,2015). Soil biodiversity supports high crop yields and high NUE (Ouni *et al*., 2014). It is thus understood that soil microorganisms are essential for plant growth (Rickson *et al.*, 2015; Kopittke *et al.*, 2019). Agricultural intensification disturbs soil microbial communities and reduces the complexity of soil ecosystems. This may occur directly through the over application of chemical inputs (mineral fertilizers and biocides), and indirectly through the depletion of SOM (Crews and Rumsey, 2017). The disturbance of soil microbial biodiversity retards nitrogen transformations in the soil. An improvement in soil management practices may improve both the SOM content and the soil microbial communities, ensuring long term soil productivity (Pimentel and Burgess, 2013).

Acidification

Acidification occurs because of human activities such as the over-application of nitrogen fertilizers (leading to acid rain) and mining activities which expose acid sulphate soils (Meng *et al*., 2019). Soil acidification hinders plant growth and decreases crop yields. Various mechanisms lead to the reduction of soil productivity due to acidification (Meng *et al*., 2019). The most important of these mechanisms is the increase in aluminum concentration in the soil, which reduces plant root growth (Kopittke *et al.*, 2019). This makes plants more susceptible to drought stress and nutritional deficiencies. It has been estimated that in China, the over-application of nitrogen fertilizers has decreased the pH of soils by 0.13 to 0.80 units since 1980 (Crews and Rumsey, 2017). The application of lime to increase soil pH is often uneconomical. Minimizing soil acidification requires improved soil and nitrogen fertilizer management practices (Rickson *et al.*, 2015; Meng *et al.*, 2019; Kopittke *et al.*, 2019).

Salinization

A third of arable land (950 million ha) globally is affected by salinization (Gomiero, 2016). This results in the loss of 0.3 - 1.5 million ha of agricultural land per year (Gomiero, 2016; Machado and Serralheiro, 2017). Salinization occurs because of irrigation with saline water and the elevation of the groundwater table, either through over-irrigation or through the removal of deep-rooted vegetation (Young *et al.*, 2015). Most vegetable crops have a low salinity threshold, ranging from 1 to 2.5 dS/m in saturated soil extracts. Agricultural intensification requires high inputs of fertilizer and irrigation, which may increase soil salinity (Machado and Serralheiro, 2017; Kopittke *et al.*, 2019). Humic substances may increase the salt tolerance of crops (Ouni *et al.*, 2014). Thus, improving SOM may improve soil fertility for long term production.

2.1.3 Managing soil organic matter

Soil organic matter is vital for maintaining and improving soil fertility, and for giving soil resilience to environmental stress (Rickson *et al.*, 2015). Increasing SOM content can reduce soil erosion and soil compaction, and improve the activity of the soil microbial communities (Gomiero, 2016). It is thus vital to consider soil management practices that can maintain or increase SOM, especially in degraded soils. The following principles should also be considered for managing SOM (Rickson *et al.*, 2015; Kopittke *et al.*, 2019);

- Intensive tillage accelerates the mineralization of organic matter, and must be minimized to increase SOM. Alternatively, conservation tillage can be adopted. In conservation tillage, the crop residues are kept on or near the soil surface, while conventional tillage incorporates crop residues into the soil – exposing them to more microbes and rapid degradation.
- Burning harvest residues deprives the soil of organic matter inputs. Instead, harvest residues should be retained in the soil and supplemented with organic amendments with known nutritional composition.
- The over application of nitrogen fertilizers and pesticides increases the rate at which SOM is mineralized and may disturb the soil microbial community. This must thus be minimized.

The factors that affect the balance between gains and losses of SOM are summarized in Table 2 (Crews and Rumsey, 2017; Kopittke *et al*., 2019).

Table 2: Factors affecting the gains and losses of SOM

Gains	Losses
Plant residues	Erosion
Minimum tillage	Intensive tillage
Harvest residue retention	Harvest residue removal
Low temperature	High temperature
High soil moisture	Low soil moisture
Mulching	Excessive application of mineral nitrogen fertilizers
Application of organic soil amendments	

2.1.4 Utilization of organic amendments in soil rehabilitation

Soil resilience is described as the soil’s ability to recover its productive state after a disturbance (Larney and Angers, 2012). Soils can restore their productive state unaided. However, certain extreme circumstances such as toxicity and a lack of nutrients may require longer periods of time for soils to restore themselves. In such circumstances, the use of soil organic amendments is crucial (Larney *et al.*, 2011; Crews and Rumsey, 2017). The following are some examples that highlight the role soil organic amendments in rehabilitating degraded soils;

- **Eroded agricultural sites**

  Studies were conducted in Alberta (Canada), where the topsoil in an agricultural field was mechanically removed to a depth of 20 cm. Mineral fertilizer, 5 cm of topsoil, and 75 t/ha of manure were each applied to improve soil productivity. The average grain yield obtained from the amended fields compared to fields with no amendment increased by 158%, 89% and 40% for manure, topsoil, and fertilizer, respectively. This was attributed to elevated levels of soil nitrate-nitrogen after manure application and the increase in SOM. The study also showed that the crop response to soil amendment increased with the extent of soil degradation (Larney *et al.*, 2017). Similar outcomes were obtained from multiple other studies (Larney *et al.*, 2011; Larney and Angers, 2012; Miller *et al.*, 2014, 2018; Celestina *et al.*, 2019).

- **Mine and quarry sites**

  The rehabilitation of mine sites requires an accumulation of organic matter and establishing a continuously growing plant community (Kuokkanen *et al*., 2019). For mine sites, it is important to establish plant cover to minimize erosion and contamination of water bodies downstream. However, it can take centuries before the mine site reaches a SOM content similar to the one it had before the mining activity. The application of organic amendments can inject enormous quantities of organic matter to initiate nutrient cycling in these sites (Hofman and Cleemput, 2004; Bulmer *et al.*, 2007). Fierro *et al.* (2000) found that the application of paper sludge supplemented with mineral nitrogen increased plant biomass on an abandoned sandpit. This was ascribed to an improvement in soil water retention and cationic exchange capacity. Zanuzzi *et al.* (2009) had similar findings using pig manure and sewage sludge on mine tailings, while Sydnor and Redente (2002) showed that mushroom compost could be used to rehabilitate a former gold mine site.

- **Forestry sites**

  Forest roads and landings (areas used during harvesting to which logs, and trees are placed before being cut and loaded into trucks for transportation) are characterized by soils with high bulk densities, fine-textured soil, and low SOM content (Larney and Angers, 2012). Sanborn *et al.* (2004) used wood chips (140 t/ha) supplemented with mineral nitrogen fertilizer (0.6 t/ha), subsoiling, and shallow tillage to rehabilitate such soils. It was found that soils amended with wood chips had the lowest bulk density and the highest growth rate of hybrid white spruce after 4 years compared to the other treatments. Bulmer *et al.* (2007) also found that a combination of tilling and the addition of wood wastes in log landings decreased the bulk density, improved the SOM and the soil water content.

The application of organic amendments may be a better rehabilitation technique compared to the use of mineral fertilizers. This is because degraded soils are often at a point where SOM content is extremely low, and the application of mineral nutrients alone is not enough to improve the physical, chemical and biological properties of the soil that are necessary for productivity (Larney and Angers, 2012). However, organic amendments are not often available in the desired quantities in degraded soils, and their application may lead to excessive nitrate leaching and pollution (Ragab, 2008).

2.2 Nitrogen fertilizers

2.2.1 Introduction

Nitrogen is a vital plant nutrient that is responsible for growth and reproduction. It constitutes up to 5% of the plant's dry mass and forms part of proteins and enzymes that are responsible for various metabolic processes that are necessary for plant survival. Chlorophyll is a nitrogen-based plant component that is responsible for photosynthesis (Scherer and Mengel, 2007). Plants thus require proportionally more nitrogen compared to other nutrients. However, a single dosage of nitrogen throughout the vegetation period is not practical because a high salt concentration in the soil can cause plasmolysis to young crops, and may lead to environmental pollution (Rajendra, 2012). On the other hand, multiple doses increase costs, and may cause physical damage to crops (Rajendra, 2012; Varadachari and Goertz, 2014). In this section, soil nitrogen dynamics, the difference between mineral and organic fertilizers, and the need for slow or controlled-release nitrogen fertilizers is discussed.

2.2.2 Soil nitrogen dynamics

Nitrogen is continuously being transferred between the soil and the atmosphere (Scherer and Mengel, 2007). Soil nitrogen is present in mineral (NH_4^+, NO_3^-) and organic forms. Plants absorb mineral nitrogen forms from the soil solution. Organic nitrogen is first mineralized by soil microorganisms before becoming plant available. Figure 1 shows an overview of the transformations of nitrogen and their link to plants and SOM. The major transformations are mineralization, immobilization, nitrification, denitrification, and volatilization (Luce *et al.*, 2011).

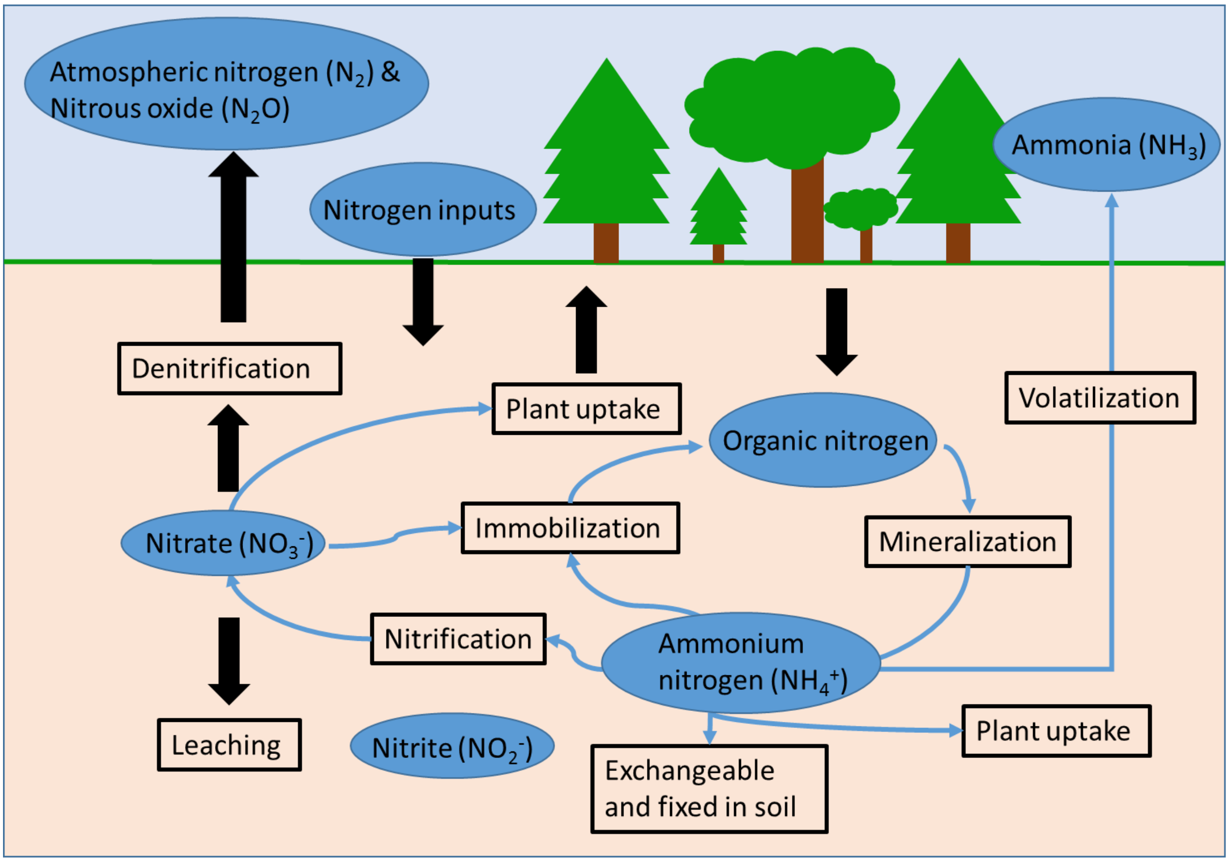


Figure 1: The nitrogen cycle, redrawn from Smil (1999)

Mineralization and immobilization

Mineralization is the microbial degradation of organic matter, producing mineral nitrogen forms. As the degradation progresses, soil microorganisms utilize carbon compounds for their energy needs and nitrogen compounds for the synthesis of proteins, amino acids, and nucleic acids (Scherer and Mengel, 2007; Talgre *et al*., 2017). If the organic substrate contains more nitrogen (and less carbon) than the microbes need, the microbes supplement their energy needs by mineralizing SOM. If the organic substrate contains less nitrogen (and more carbon), the microorganisms supplement their nitrogen needs by absorbing mineral nitrogen forms in the soil. This process is called immobilization. Immobilization reduces the amount of plant available nitrogen in the soil (Galloway, 1998).

The plant-microbe interaction for nitrogen may be summarized as follows (Xi, 2015);

1. Plants absorb mineral nitrogen forms. Organic nitrogen is made plant available through mineralization by soil microorganisms.

2. The amount of plant available nitrogen in the soil is determined by the mineralization-immobilization turnover.
3. Soil microorganisms are better competitors for mineral nitrogen in the soil because they have a higher surface area to volume ratio compared to plants roots, and they have a faster growth rate.

Mineralization and immobilization processes occur concurrently during the degradation of organic residues. The mineralization-immobilization turnover depends on the carbon-to-nitrogen ratio (C/N) of the organic residues. The critical range of the C/N ratio is between 25 and 30 (Fischer and Schiene, 2002; Luce *et al*., 2011). Organic residues with high C/N ratios (>30) cause a net immobilization of nitrogen in the soil, while those with low C/N ratios (<25) cause a net mineralization of nitrogen in the soil (Scherer and Mengel, 2007). The C/N ratio also determines the rate of decomposition of organic residues, and thus determines the rate at which nitrogen is released. It is thus a critical parameter in the selection of organic soil amendments. Table 3 below shows the typical C/N ratios of organic material that is expected to cause a net mineralization and a net immobilization of nitrogen in the soil (Talgre *et al*., 2017).

Table 3: Typical C/N ratios of dried organic material

Net mineralization of Nitrogen		**Net immobilization of Nitrogen**	
Material	**C/N**	**Material**	**C/N**
Manure	15:1	Sugar cane tops	80-100:1
Poultry litter	7-11:1	Eucalyptus sawdust	500:1
Humus	10-15:1	Bagasse	120:1
Urine	15-19:1	Peat	30:1
Hay	13:1	Composted pine bark	30-40:1

Nitrification and denitrification

Nitrification is the aerobic conversion of ammonium nitrogen into nitrate nitrogen. This is accomplished in two steps by different autotrophic bacterial species. *Nitrosomonas* bacteria first convert the ammonium cation into nitrite (NO_2^-), *Nitrobacter* bacteria then convert the nitrite ion into nitrate (NO_3^-) (Luce *et al*., 2011; Gul and Whalen, 2013). Unlike ammonium ions, nitrate ions are not fixed by clay minerals, but are mobile in the soil solution. This makes them susceptible to leaching, which leads to eutrophication. Ammonium nitrogen may also be converted to ammonia gas which tends to escape into the atmosphere (Khan *et al.*, 2017). Alkaline, moist, and warm soils enhance ammonia volatilization. Denitrifying microbes can reduce nitrate ions (producing nitrogen gas) to meet their oxygen needs in anaerobic conditions; this process is called denitrification. Both nitrification and denitrification processes reduce the amount of plant available nitrogen in the soil (Smil, 1999; Waqar *et al.*, 2014), with a concomitant release of nitrogen oxides (Basosi *et al*., 2014).

2.2.3 Nitrogen fertilizers: Definition and classification

Nitrogen is removed from the soil when plants are harvested, through nitrate leaching and denitrification, and through ammonia volatilization. Immobilization also reduces the amount of plant available nitrogen. A fertilizer is a product that increases the quantity of plant nutrients in the soil or improves the chemical and physical properties of the soil to enhance plant growth, increase crop yield, and improve crop quality (McGrath *et al.*, 2014; Rong *et al*., 2016). They are classified into two categories as follows (Scherer and Mengel, 2007);

- **Organic fertilizers** are obtained from organic wastes, such as animal husbandry (manure), plant decomposition products (composts, peat) and waste treatment (composted garbage, sewage sludge).
- **Mineral fertilizers** are produced through the industrial fixation of nitrogen. Atmospheric nitrogen is reacted with hydrogen under high pressure to form ammonia which can be used directly as a fertilizer or be processed further to produce nitrogen salts such as urea, ammonium nitrate, and ammonium sulphate.

2.2.4 Organic fertilizers

The fundamental contention for the utilization of organic fertilizers is the preservation of natural nutrient resources through systematic recycling (Crews and Rumsey, 2017). Organic fertilizers are a significant part of organic agriculture because they increase SOM levels, which is essential for the remediation and restoration of degraded soil (Ndukwe, 2012). However, they usually have a low nutrient content, and are thus required in massive quantities to meet the desired nutrient application rates (Ragab, 2008; Li *et al.*, 2014). Furthermore, their application rate is limited by their pollution load (Li *et al*., 2021). Organic fertilizers need to be mineralized by soil microorganisms before the nutrients they contain are released and become available for plant uptake. The limitations of organic fertilizers may be summarised as follows (Chen, 2006; Timsina, 2018;Kopittke *et al.*, 2019);

- Low and variable nutrient contents
- Nutrient release depends on soil microbial activity, which is sensitive to soil moisture and temperature. As a result, nutrient release may be too slow to meet plant requirements over a short period.
- Prolonged application may lead to nutrient or heavy metal accumulation.
- Their application may not be feasible on a large scale
- Profit margins in commercial farming provide little motivation for a long-term investment in soil, especially if it comes with a sacrifice of short-term profitability.

2.2.5 Mineral fertilizers

Mineral fertilizers are responsible for the production of 50% of global food production, and current agricultural intensification measures are dependent on mineral fertilizers because crop yields have a rapid response to their application (Crews and Rumsey, 2017). However, mineral fertilizers have led to severe degradation of agricultural soil, and their efficiency has decreased to less than 50% due to a decline in soil productivity (Johnson and Raun, 2003; Kopittke *et al.*, 2019). In contrast to organic fertilizers, mineral fertilizers have a high and constant nutrient content, and can be formulated to the desired ratio to meet plant requirements. However, they have the following limitations (Bouwman, 1996; Scherer and Mengel, 2007);

- Mineral fertilizers do not often contain micronutrients
- Mineral fertilizers do not add organic matter to the soil directly to support soil microbial communities.
- Mineral fertilizers dissolve easily and release nutrients much faster than plants can absorb them. This may result in harmful health and environmental effects.
- Prolonged application of mineral fertilizers can cause soil acidification, disturb soil micro biodiversity and lead to the release of greenhouse gases.

Health effects

The presence of enormous quantities of mineral nitrogen forms in the soil results in nitrogen losses that can be harmful to both human life and the environment (Waqar *et al.* 2014). High nitrate content in food crops and drinking water (>10 mg/ℓ) (especially obtained from groundwater reserves) can pose health risks, especially to infants (Shaviv and Mikkelsen, 1993). Nitrates are converted to nitrites in the blood which can affect the ability of hemoglobin to release oxygen to body cells. This can lead to a fatal condition called methemoglobinemia. Other health conditions associated with the consumption of high quantities of nitrates include gastric cancer, goitre and brain cancer (Johnson, 2019).

Environmental effects

The global warming potential of nitrous oxide (N_2O) is multiple times more prominent than that of carbon dioxide. Nearly 42% of all nitrous oxide emissions are derived from agriculture (Bouwman, 1996). Nitrogen oxides are produced through nitrification and denitrification processes in the soil. Improved management of fertilizers can reduce these emissions. Furthermore, nitrogen losses through nitrate leaching can cause eutrophication (Savci, 2012; Basosi *et al.*, 2014).

2.2.6 Organic vs mineral fertilizers

While mineral fertilizers provide high nutrient contents and rapid yield increases, organic fertilizers provide SOM directly, which improves soil quality and enhances soil fertility. Neither fertilizer type can provide both properties on its own. Applying combinations of these two fertilizer types can maximize crop yields, increase soil fertility, and sequester carbon dioxide in agricultural soils. For example, Chivenge *et al.*, (2010) reported that using a combination of organic fertilizers and mineral fertilizers had a higher yield increase on maize compared to organic fertilizers and mineral fertilizers applied separately. Rong *et al.*, (2016) found that combining organic and mineral fertilizers produced the highest rate of soil organic carbon accumulation compared to the application of these fertilizers separately. Similar results have been reported by multiple other researchers (Chen, 2006; Bhattacharyya *et al.*, 2008; Tana and Woldesenbet, 2017; Bistgani *et al*., 2018; Roba, 2018; Gram *et al*., 2020).

2.3 Lignin chemistry

2.3.1 Significance and occurrence of lignin

For millennia, lignocellulosic biomass (plant dry matter), has served humankind as construction material, as fuel for heat, and as a raw material for producing pulp and paper products (Himmel *et al*., 2007). It is a renewable resource that can be used to sustainably produce a wide range of products (Beckham, 2018). Most of the plant biomass is contained in cell walls, which are composed of cellulose, hemicellulose, and lignin (Datta *et al.*, 2017).

Cellulose is a straight-chain polymer composed of glucose. Hemicellulose is a branched polymer composed of xylose, arabinose, galactose, mannose and glucose (Dixon *et al.*, 2019). Lignin is composed of three different phenyl propane units. The units; p-courmaryl, coniferyl, and sinapyl alcohols are the precursors of the monomers of lignin, namely, p-hydroxyphenyl (H), guaiacyl (g), and syringyl (S) units (Boerjan *et al.*, 2003; Dixon *et al.*, 2019), respectively (shown in Figure 2). Lignin is formed through the enzymatic dehydrogenation of these monomers, which randomly forms C-C and C-O linkages, resulting in the heterogeneous structure of lignin (Beckham, 2018).

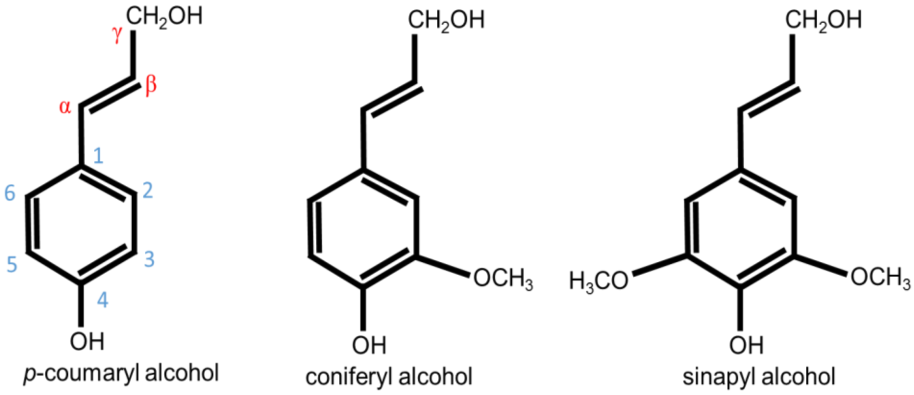


Figure 2: Monomers of lignin, redrawn from Beckham (2018)

Lignin binds the cellulose fibres together, giving lignocellulosic biomass its strength and rigidity (Gul and Whalen, 2013). It also enables xylem tissues to transport water, nutrients, and metabolites, and resist biological attack from microorganisms (Pedersen *et al.*, 2005). However, both the quantity and composition of lignin varies between plant species, between different morphological parts of the same plant, and with the environmental conditions in which the plant grows (Calvo-Flores, 2015). Lignin constitutes 25% – 35% of softwood biomass, 20% – 25 % hardwood biomass, and 15%

– 25% of herbaceous plant biomass (Calvo-Flores, 2015; Beckham, 2018). Herbaceous plants contain all three monolignols, hardwood lignin contains G and S units, and softwood lignin mainly consists of G units. The content of lignin in biomass increases in the following order: grasses < hardwood < softwood (Le Roy *et al*., 2017 ;Weng and Chapple, 2010; Azadi *et al*., 2013).

2.3.2 Structure

Inter-unit linkages

The monomers of lignin are linked by aryl ether bonds (β-O-4, α-O-4, 4-O-5) and covalent carbon-carbon bonds (5 - 5, β - 5, β - 1, β - β). More than two thirds of inter-unit linkages in lignin are ether linkages (Rodrigue*z et al.*, 1996; Beckham, 2018). The most abundant linkage is the β-O-4, accounting for about 50% of the inter-unit linkages present in lignin. Hardwoods contain more β-O-4 linkages than softwoods (Azadi *et al.*, 2013). The cleavage of this linkage finds importance in pulping processes, both because of its abundance and because it is a thermodynamically labile linkage and is thus easy to cleave (Santos *et al.*, 2013; Calvo-Flores, 2015).

Funtional groups

Lignin consists of hydroxyl (phenolic and aliphatic), methoxyl, and carbonyl groups in its structure. Softwood lignin contains more hydroxyl and carbonyl groups compared to hardwood lignin (Calvo-Flores, 2015). However, hardwoods have a higher methoxyl group content compared to softwoods. This is due to the greater abundance of S units in hardwood lignin. Phenolic hydroxyl groups are directly involved in the polymerization of lignin (Beckham, 2018). Technical lignins contain more phenolic hydroxyl groups compared to native lignin (Vishtal and Kraslawski, 2011; Chio *et al*.,2019). This is because the cleavage of β-O-4 linkages during pulping results in the formation of new phenolic hydroxyl groups. This functional group improves the degree of brightness and stability of pulp (Beckham, 2018). The abundance of oxygen rich functional groups in lignin also enables it to chelate metal ions, increasing its potential for agricultural applications (Abaecherli and Popa, 2005; Chen *et al*., 2020).

Lignin carbohydrate complexes

Lignin is inherently linked to the carbohydrates present in lignocellulose, forming complexes know as lignin carbohydrate complexes (LCC). Four types of LCC linkages have been identified; benzyl ether, γ – ester, conjugate γ – ester, and phenyl glycoside (Beckham, 2018). Some LCC may be formed during chemical pulping. The cleavage of beta-aryl bonds (through the dominant pulping reactions) can result in the formation of covalent ether bonds between lignin and carbohydrates (Boerjan *et al.*, 2003). A lignin or carbohydrate sample obtained from lignocellulose is never free from the other component. There is always a certain amount of carbohydrates linked to lignin in a sample (Ragab, 2008). This may present a challenge in the valorisation of lignin, especially in applications where the presence of carbohydrates can yield undesired products.

2.3.3 Delignification of biomass

The goal of pulping is to remove enough lignin from biomass to allow for the separation of cellulosic fibers from one another. The resulting pulp is then used to manufacture pulp and paper related products. The extent of delignification determines the quality of the pulp produced, and the product range that can be produced from it (Ek *et al.*, 2009; Suhr *et al.*, 2015).

Biomass delignification is accomplished through two main chemical modifications; fragmentation, and solubilisation (Matsushita, 2015; Aro and Fatehi, 2017). Fragmentation occurs through the cleavage of inter-unit linkages. This modification targets ether bonds due to their lower stability compared to covalent bonds. Solubilisation is accomplished through the introduction of hydrophilic groups into the lignin molecule and its fragments (Ek *et al.*, 2009). This increases the solubility of lignin in the cooking liquor, aiding its removal. The cleavage of inter-unit linkages is accompanied by the formation of hydrophilic phenolic hydroxyl groups. This also increases the solubility of the lignin and its fragments (Boerjan *et al.*, 2003; Calvo-Flores, 2015).

Delignification can be achieved through the action of bisulphite ions (HSO_3^-, sulphite pulping), hydroxide ions (OH^-, soda pulping) or a combination of hydroxide and hydrosulphide ions (OH^-, HS^-, kraft pulping). As a result, each pulping process or technique imparts a unique chemical modification to the lignin structure (Ek *et al.*, 2009; Suhr *et al*, 2015; Beckham, 2018).

The main chemical reactions that occur during chemical pulping are;

- Cleavage of aryl inter-unit linkages
- Demethylation

- Condensation

The mechanism and the extent to which each of these three reactions occur differs with each pulping technique. Technical lignins have distinct differences compared to their natural lignins (Vishtal and Kraslawski, 2011). They also differ from technical lignins obtained from other pulping processes (Boerjan *et al.*, 2003). The polymerization degree of the residual carbohydrates, and the degree of carbohydrate removal also differs depending on the extent of delignification achieved and the delignification process used (Calvo-Flores, 2015). Technical lignins usually have higher contents of phenolic hydroxyl groups and carboxylic acid groups, and lower contents of aliphatic hydroxyl groups and β-O-4 linkages compared to natural lignins (Ek *et al.*, 2009).

Kraft pulping

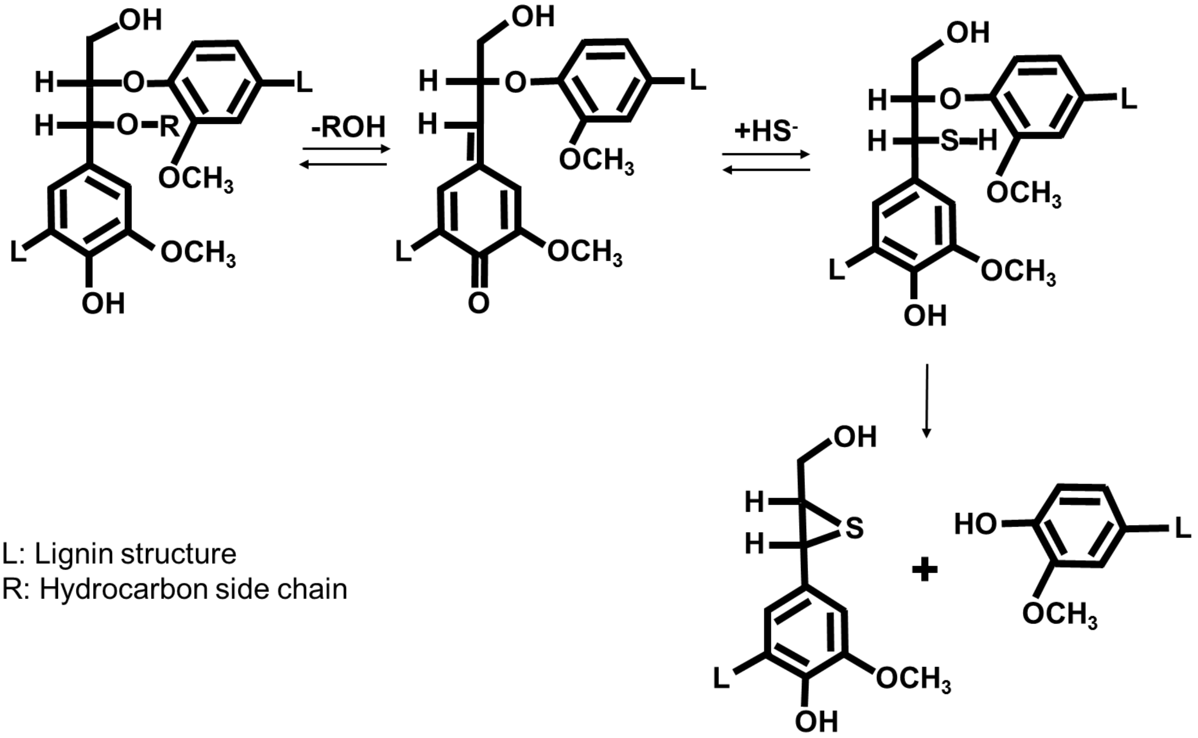
Kraft pulping is currently the dominant pulping process. This is because it produces stronger pulps and has an efficient chemical recovery process. Sodium hydroxide and sodium sulphide are used as the pulping reagents in strong alkaline conditions (Ek *et al.*, 2009; Santos *et al.*, 2013; Suhr *et al*., 2015). The cleavage of β-O-4 linkages is facilitated by the hydrosulphide ion. However, this modification depends on the type of moiety (free or etherified phenolic group) present at the C_4 position para to the propane side chain (Matsushita, 2015).

a) Phenolic structures

The reactions (see Figure 3) involved in the degradation of phenolic structures in Kraft pulping can be summarised as follows (Ek *et al.*, 2009; Matsushita, 2015);

1. Phenolic benzyl alcohol forms an equilibrium with its quinone methide in alkaline conditions. This occurs through the removal of the OR group from $C_{\alpha.}$
2. The hydrogen sulphide ion is incorporated on C_α and restores the aromaticity of the phenyl propane unit.
3. The cleavage of the β – aryl bond by hydrogen sulphide, forming episulphide and a phenolic structure. The episulphide is unstable and may lead to the release of elemental sulphur and a coniferyl alcohol fragment. Reactions 1 – 3 are shown in

Figure 3. Elemental sulphur is quickly converted to thiosulphate by hydrogen sulfide ions (Calvo-Flores *et al.*, 2015).



L: Lignin structure
R: Hydrocarbon side chain

Figure 3 : A simplified schematic figure of one of the mechanisms for the cleavage of phenolic β-O-4 linkages during Kraft pulping (Ek et al., 2009)

4. Alkali promoted elimination of terminal hydroxyl groups from Cγ, as shown in Figure 4 below.

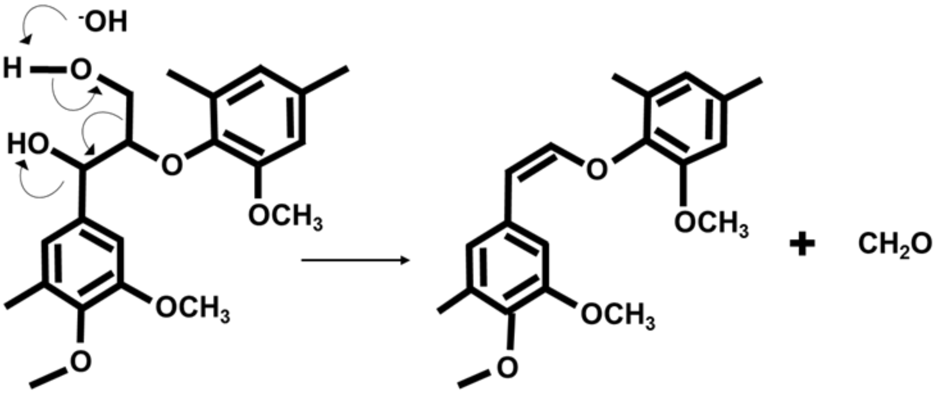


Figure 4 : Elimination of terminal hydroxyl groups from Cγ (Matsushita, 2015)

5. Demethoxylation (occurs in both phenolic and etherified structures) via a hydrosulphide ion attack on methoxyl groups. This produces a phenolic structure

and a methyl mercaptan. The methyl mercaptide ion may also effect a nucleophilic attack on other methyl groups, forming dimethylsulfide (Matsushita, 2015).

a) Non-phenolic structures

The cleavage of non-phenolic β-O-4 structures occurs through the ionization of Cα and Cγ hydroxyl groups (Ek *et al.*, 2009). This produces an unstable epoxide and a phenolic lignin structure, leading to further fragmentation. Unlike phenolic β-O-4 linkages, the cleavage of non-phenolic β-O-4 linkages is dependent on the hydroxide ion concentration. This cleavage is much slower compared to that of phenolic β-O-4 structures. Figure 5 below shows a simplified degradation of non-phenolic structures. A similar reaction mechanism takes place in the degradation of non-phenolic structures during soda pulping (Matsushita, 2015; Beckham, 2018).

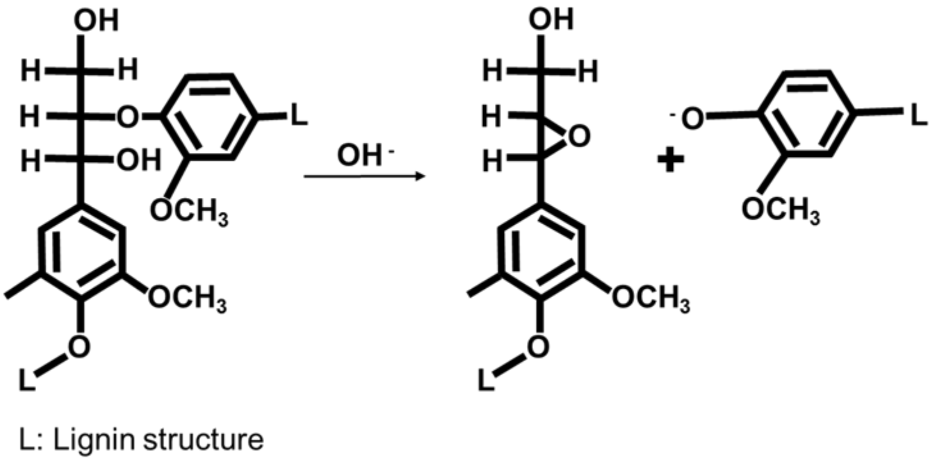


Figure 5 : A simplified schematic figure of one of the mechanisms for the cleavage of etherified β-O-4 linkages (Ek *et al.*, 2009)

Soda pulping

Soda pulping is used for the chemical pulping of non-wood material such as bagasse and wheat straw. Sodium hydroxide is the main pulping reagent used in this process. Sometimes anthraquinone is used to increase the rate of delignification (Ek *et al.*, 2009; Suhr *et al.*, 2015).

a) Phenolic structures

Similar to kraft pulping, phenolic groups are ionized in strong alkaline conditions, forming a quinone methide. However, an episulphide structure is not formed due to the absence of hydrogen sulphide (Ek *et al.*, 2009; Matsushita, 2015). Instead, the quinone methide undergoes the following reactions, as shown in Figure 6;

1. Reduction through the addition of a hydroxide ion
2. Condensation with other lignin fragments
3. Elimination of $^{-}CH_2O$ group, forming a stable enol ether

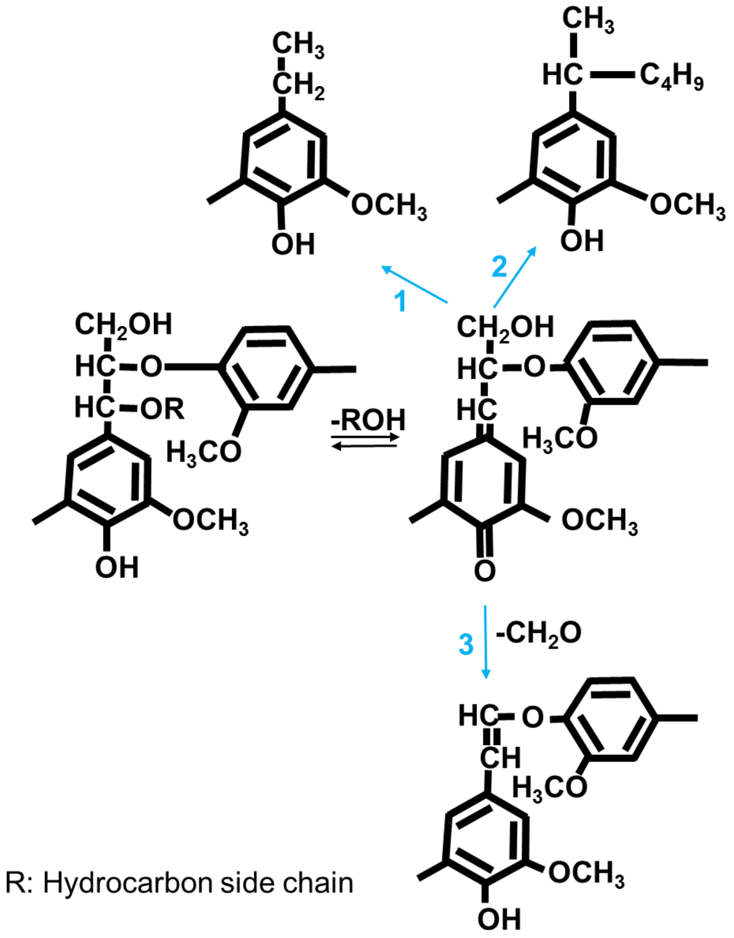


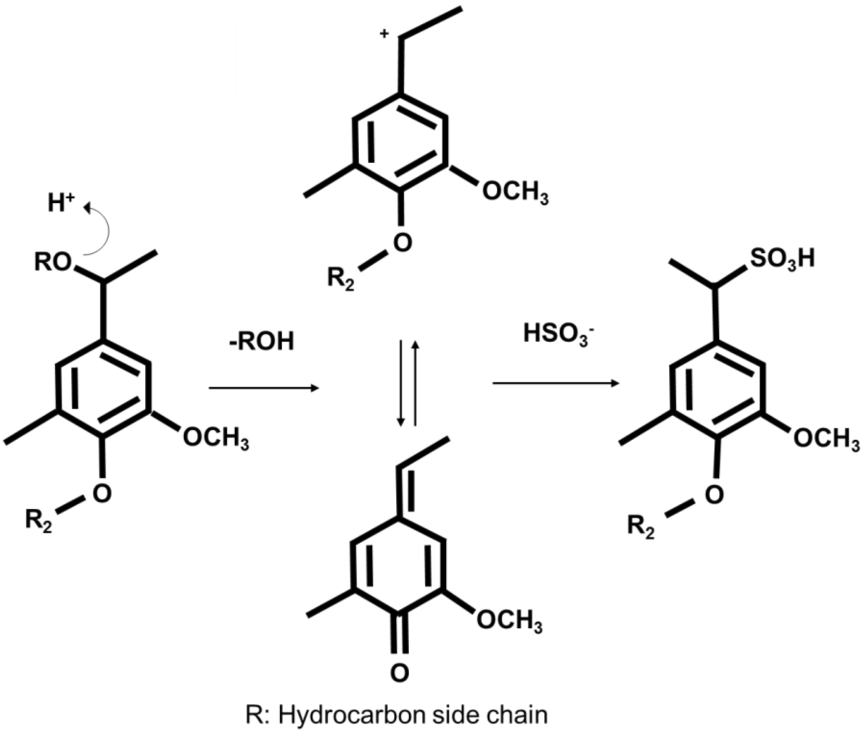
Figure 6: Reactions of a quinone methide structure in the absence of hydrogen sulfide (Calvo-Flores *et al.*, 2015; Holm, 2018)

Sulphite pulping

Sulphite pulping offers flexibility in terms of operating conditions and the quality of the pulp produced. It allows for the production of several types of pulps for an extensive scope of utilization (Suhr *et al.*, 2015; Aro and Fatehi, 2017).

In this process, delignification is accomplished by the action of the bisulphite (HSO_3^-) and sulphite ions (SO_3^{-2}). It can be used with a wide range of counter ions, namely; calcium, sodium, magnesium, or ammonium ion. Most sulphite pulping processes use strongly acidic conditions (pH less than 5), except for neutral sulphite semi-chemical pulping (NSSC), which is carried out at pH 5 – 7 (Ek *et al.*, 2009; Matsushita, 2015). Delignification is accomplished through two reactions: sulphonation and hydrolysis. Acid pulping occurs through both phenolic and etherified structures, while neutral conditions target phenolic structures. The reaction steps involved in sulphite pulping can be summarised as follows (Ek *et al.*, 2009; Matsushita, 2015; Aro and Fatehi, 2017);

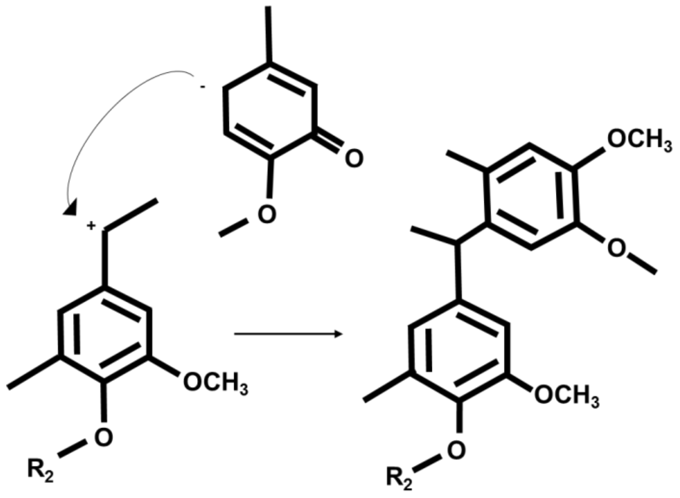
1. Under acidic conditions, a quinone methide structure is formed through the protonation and cleavage of the alpha-aryl linkage (hydrolysis reaction).
2. The bisulphite ion is incorporated at the C_α, forming benzyl sulphonic acid (sulphonation), as shown in Figure 7.



R: Hydrocarbon side chain
R_2: H or Hydrocarbon side chain

Figure 7: Acid sulphonation (Matsushita, 2015; Aro and Fatehi, 2017)

3. In acidic conditions, condensation takes place between C_α and C_6 of another molecule (see Figure 8). This is an undesired reaction because it prevents the sulphonation of the lignin fragment (Ek et al., 2009).



R_2: H or Hydrocarbon side chain

Figure 8 : Condensation reaction occurring in acidic pulping

4. Under neutral conditions, the electrophilic nature of the alpha sulphonic group leads to a nucleophilic attack on the Cβ by the sulphite ion, cleaving the Cβ –aryl bond. This may result in the introduction of two sulphonate groups in the same phenyl propane side chain. This also releases a new phenolic fragment which can also undergo sulphonation (Ek *et al.*, 2009; Matsushita, 2015). Figure 9 shows the reactions that occur during neutral sulphite pulping.

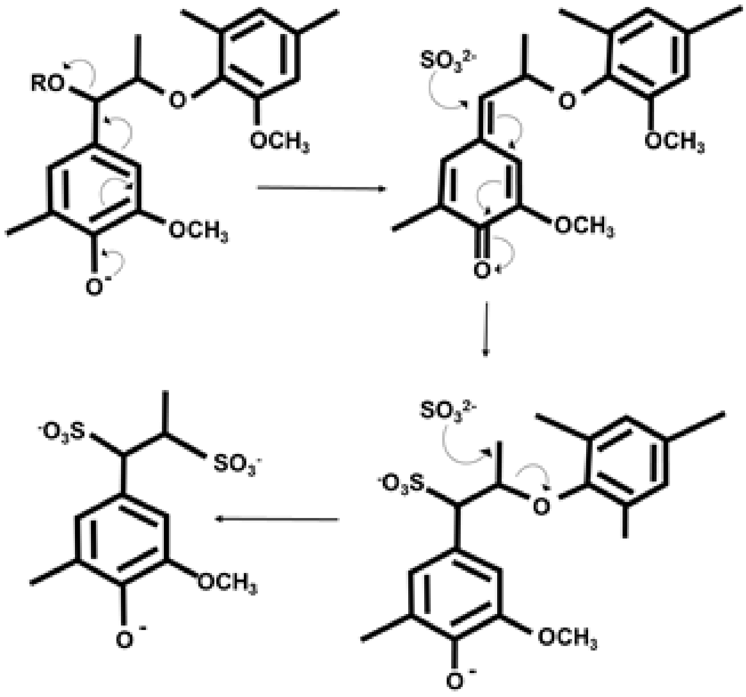


Figure 9 : Neutral sulphite sulphonation reaction (Aro and Fatehi, 2017)

Structural differences between technical lignins

Different pulping methods and conditions impart distinct structural modification in lignin. This in turn influences the physical properties of each lignin type, which affects the applications for which each lignin can be used (Beckham, 2018).

Lignosulphonates have a large quantity of sulphonate groups, which makes them water soluble at any pH. They also have unique colloidal properties that allow them to be used as dispersing agents (Abaecherli and Popa, 2005), binders for drilling agents (Zhang *et al.*, 2014), adhesives and cement additives (Zhang *et al.*, 2020). The sulphur and ash contents of this technical lignin is generally higher than kraft and soda lignins (Ek *et al.*, 2009).

Kraft lignins contain a high quantity of phenolic hydroxyl groups due to the cleavage of beta aryl bonds during pulping (Vishtal and Kraslawski, 2011; Matsushita, 2015). As opposed to kraft lignin and lignosulphonates, soda lignin does not contain sulphur. It does, however, contain vinyl ether groups in its structure (Santos *et al.*, 2013; Souza *et al.*, 2020). Kraft lignin and soda lignin are usually more depolymerized during the pulping process. As a result, bacteria can degrade phenolic compounds from these lignins. Technical lignins obtained from pulping processes with a lower degree of delignification are more etherified and polymerized, making them recalcitrant to bacterial degradation (Beckham, 2018). Table 4 summarizes the main differences between these three lignin types.

Table 4 : Differences between lignosulphonate, kraft and soda lignin (Vishtal and Kraslawski, 2011; Beckham, 2018; Souza *et al.*, 2020)

	Lignosulphonates	**Kraft**	**Soda**
Functional groups	Fewer phenolic, hydroxyl, carboxyl and catechol groups compared to Kraft and Soda lignin	Phenolic, hydroxyl, carboxyl and catechol groups	Phenolic, hydroxyl, carboxyl and catechol and vinyl ether groups
Sulphonate groups (per phenylpropane unit)	0.4 – 0.5	-	-
Organic sulphur (%)	4 – 8	1 – 1.5	-
Solubility	Water	Alkali, acetone, DMF, Methyl cellosolve	

2.3.4 Oxidative degradation of lignin

Both natural humus formation and ammoxidation (discussed further in section 2.5) involve an oxidative degradation of organic substrates, including lignin (Fischer and Schiene, 2002). It is thus important to understand the structural modification that occurs during the oxidative degradation of lignin, and the functional groups that are most reactive to oxidation.

Rationale for oxidation

The formation of lignin is an overall reductive process. Thus, oxidation can be an efficient method of disrupting the molecular structure of lignin (Beckham, 2018). The compact and electronically dense structure of lignin hinders its effective depolymerisation (Junghans *et al.*, 2020). Even though the monolignols are linked by ether (C-O-C) and carbon-carbon (C-C) bonds, the electron density of the monolignols has a significant contribution to the compactness and stability of lignin. Oxidation is a dominant lignin depolymerisation and degradation process used in chemical pulp bleaching to decrease the amount of lignin that remains and improve the brightness of pulp (Ma *et al.*, 2017; Beckham, 2018;

Vangeel *et al.*, 2018). The oxidation of lignin in alkaline media is used for the production of vanillin (food flavouring) and syringaldehyde (precursor for various chemical and pharmaceutical products). The formation of humic substances in the soil also occurs through the oxidation of lignin by soil microorganisms (Rodriguez *et al.*, 1996).

Reactivity of lignin structures

Molecular structures that contain phenolic hydroxyl groups have the highest reactivity to oxidation, followed by molecular structures that contain methoxyl groups. The reactivity of lignin monomers to oxidation thus increases with increasing degree of methoxylation (S > G > H) (Northey, 2001; Lücke *et al.*, 2004; Imai *et al.*, 2008). The relative reactivities of different lignin-related structures were summarised by Northey (2001) as shown in Figure 10.

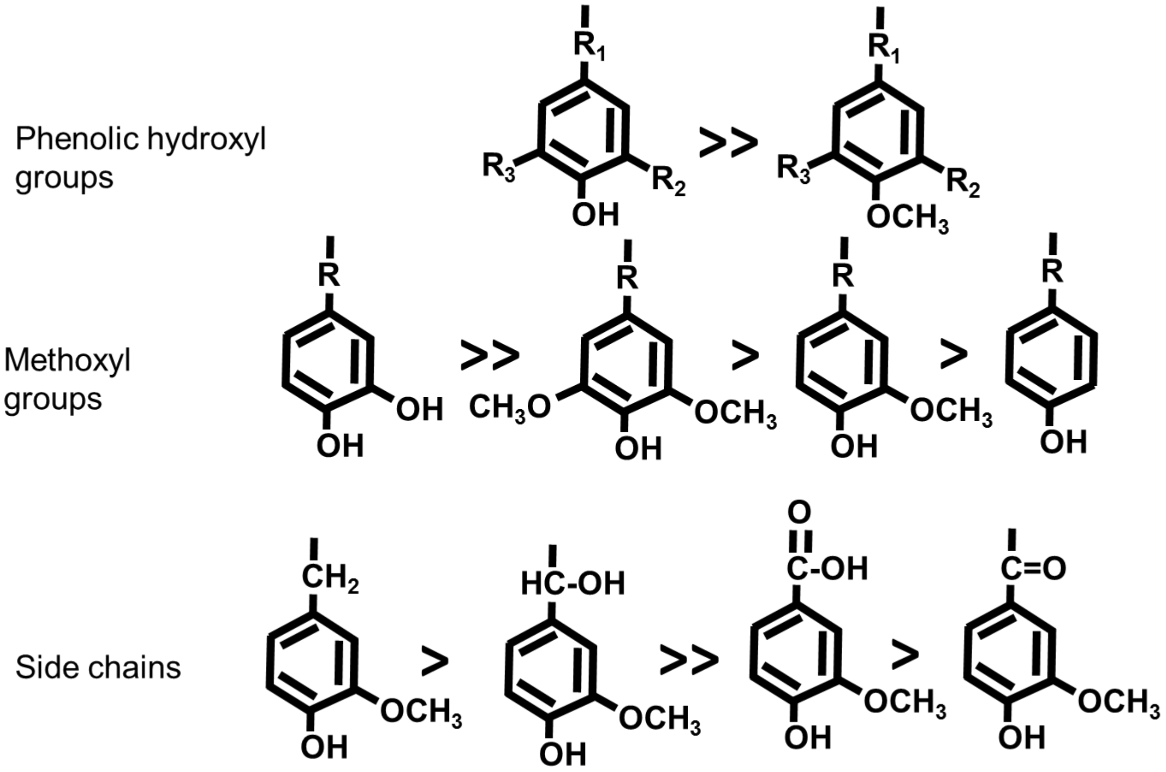


Figure 10 : Reactivity of lignin structures, redrawn from Northey (2001)

Structural modifications during lignin oxidation

The oxidation of lignin involves the following structural modifications;

1. Oxidation of side chains
2. Oxidation of aromatic rings

a) Oxidation of side chains

The oxidation of side chains leads to the incorporation of oxygen-rich functional groups onto the propanyl side chains. This impairs the π-π interactions among phenyl propane units, which leads to fragmentation (Ma *et al.,* 2017; Junghans *et al.*, 2020). Figure 11 shows the oxidation of side chains in model monomeric compounds

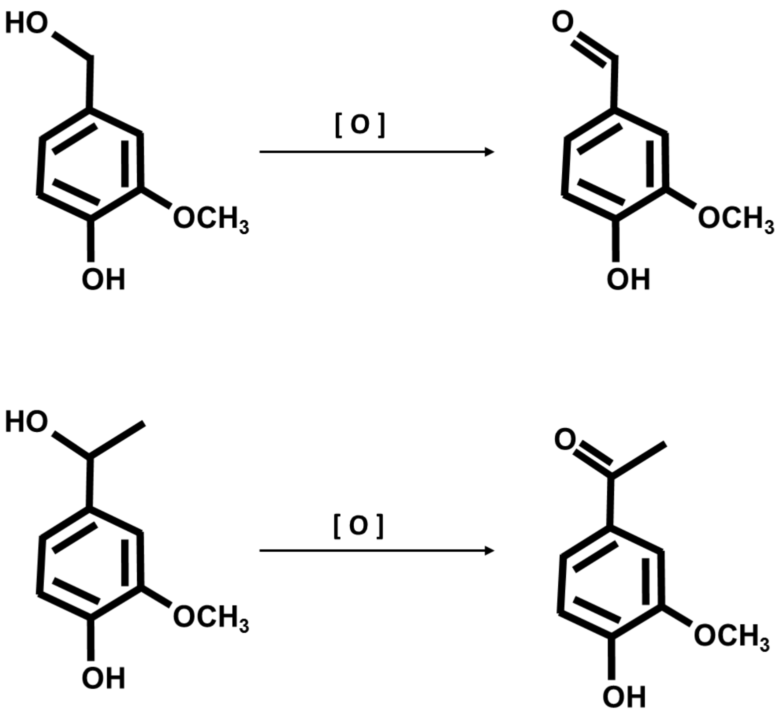


Figure 11: Oxidation of side chains. Redrawn from Beckham (2018)

b) Oxidation of aromatic rings

Strongly oxidative conditions lead to the oxidation of aromatic rings. These reactions often occur in delignification and bleaching (Beckham, 2018). Aromatic ring cleavage disturbs the electron density of lignin, converting it into open chain structures. This forms benzoquinones, dicarboxylic acids, and subsequent ring cleavage products (He *et al.*, 2017; Ma *et al.*, 2017), as shown in Figure 12 . In the soil, white rot fungi degrade phenolic lignin structures through oxidative one electron mechanisms, starting with phenolic aromatic rings (Thevenot *et al.*, 2010; Asina *et al.*, 2016; Datta *et al.*, 2017; Ma *et al.*, 2017).

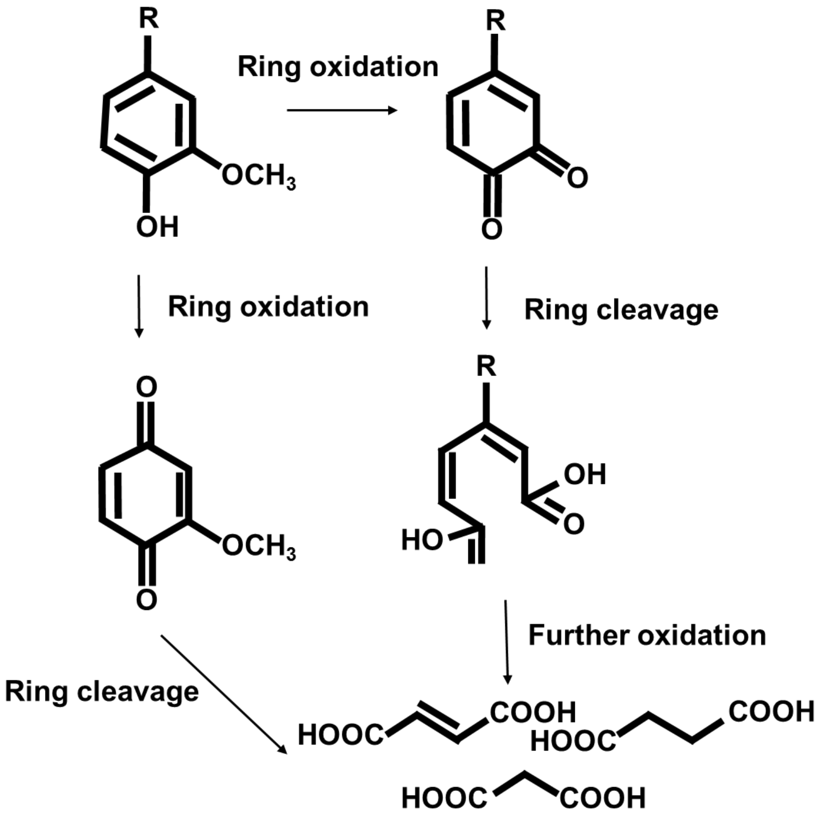


Figure 12 : Oxidation of aromatic rings. Redrawn from Beckham (2018)

2.3.5 Enzymatic degradation of lignin in the soil

At the end of plant life, some plant matter returns to the soil for decomposition. The ability of soil microorganisms to degrade lignin is an important evolutionary trait that returns most of the carbon fixed by plants during their growth, and contributes to the formation of humic substances in the soil (Datta *et al.*, 2017; Beckham, 2018). Degraded lignin fragments serve as the starting material for the formation of humus – the component of the soil that is vital for soil fertility (Fischer and Schiene, 2002; Abaecherli and Popa, 2005; Gomiero, 2016; Solaiman *et al.*, 2019).

Lignin degrading baciomyecetes are classified into two; white-rot and brown-rot fungi (Reid, 1995; Datta *et al.*, 2017). Some species of bacteria are also able degrade lignin, but white rot fungi are the most efficient lignin degrading microorganisms. Brown rot fungi degrade lignin through non-enzymatic oxidation reactions, and partially oxidize lignin through aromatic ring demethylation. White-rot fungi are able to completely mineralize lignin into carbon dioxide and water (Calvo-Flores, 2015; Asina *et al*, 2016). Lignin degrading microorganisms produce the following ligninolytic enzymes; laccase, lignin peroxidase, manganese peroxidase, versatile peroxidase, and dye-decolorizing peroxidases. Lacasses are not substrate specific, and can oxidize a wide range of substrates including polyphenols, benzenethiol and aromatic amines (Rodriguez *et al.*, 1996; Datta *et al*., 2017; Liu *et al*., 2020).

2.4 Soil organic matter

Soil is composed of different materials. This includes mineral matter, organic matter, water and air contained within the pore spaces. Soil organic matter (SOM) consists of plant and animal residues at different stages of decomposition (Bot and Benites, 2005; Fageria, 2012). It is a crucial component of the soil because it improves the physical, chemical, and biological properties of the soil that promote plant growth. Productive agricultural soils contain 100 tons of SOM per hectare. This is about 4% to 5% of topsoil weight (Pimentel and Burgess, 2013). A large fraction of SOM is carbon (~58%), although it also contains oxygen, hydrogen, nitrogen, phosphorus, sulphur, and other elements. More than 90% of soil nitrogen, and 25% to 50% of soil phosphorus, are contained in SOM (Nourbakhsh, 2006; Gomiero, 2016). The quantity of SOM in topsoil is determined by the balance between inputs (organic residues) and outputs (microbial decomposition) (Plaza and Senesi, 2009; Gomiero, 2016).

The components that make up SOM (and their relative weight distribution) can be grouped into the following three categories (Huang and Hardie, 2009);

1. Fresh organic residues (<10%), and microbial organisms (<5%).
2. Detritus or active organic matter (33% - 50%) – this is organic matter at the intermediate stage of decomposition.
3. Humus, the final product of decomposition (33% - 50%) –this is the stable fraction of organic matter.

Humus formation

It is estimated that only 0.7% of the active organic matter (fresh organic residues and detritus) that enters the soil annually is converted to humus (Plaza and Senesi, 2009). In the initial stages of decomposition, organic residues that decompose easily (sugars, starches, hemicelluloses, amino acids) and some recalcitrant compounds such as cellulose are decomposed (Huang and Hardie, 2009). Carbon dioxide, organic acids, volatile compounds, and other incompletely oxidised compounds are produced. In the next stage, organic intermediates, the remainder of cellulose, newly formed biomass, and some lignins are decomposed. In the last stage, the more recalcitrant components (lignins) are decomposed, gradually forming compounds that are more recalcitrant to decomposition, i.e., humus, which may persist in the soil for millennia (Pimentel and Burgess, 2013; Rickson *et al*., 2015).

The ease of decomposition of organic compounds can be ranked as follows (Huang and Hardie, 2009);

1. Sugars, starches, and simple proteins
2. Crude proteins
3. Hemicellulose
4. Cellulose
5. Fats, waxes
6. Lignins and phenolic compounds

Rapid decomposition
↕
Slow decomposition

Humus is divided into three components: fulvic acids, humic acids and humins. Humins are insoluble in alkali. Fulvic acids and humic acids are alkali soluble, but humic acids can be acid precipitated due to their larger molecular mass compared to fulvic acids. Three main theories have been proposed for the formation of humus in the soil: the lignin theory, the polyphenol theory, and the sugar-amine theory (Abaecherli and Popa, 2005; Sen *et al.*, 2020).

The lignin theory considers lignin as the precursor of humic substances. It is postulated that lignin is incompletely decomposed by soil microorganisms. Lignin undergoes various structural modifications during the decomposition, including demethoxylation, formation of O-hydroxyphenols, and side chain oxidation which forms carboxylic acid groups (Shah *et al.*, 2018; Zanella *et al.*, 2018). The O-dihydrobenzene fragments (from lignin demethylation) are oxidised to quinones which undergo condensation reactions with amino compounds. The modified lignin structures are decomposed further to form humic acids and then fulvic acids (Plaza and Senesi, 2009). This theory is upheld by the observation that lignin is chemically similar to humic substances.

These similarities include (Abaecherli and Popa, 2005; Huang and Hardie, 2009);

- Recalcitrance to microbial degradation
- Both compounds contain methoxyl groups

- Humic acids are similar to oxidized lignins
- Both compounds are partially soluble in alkali, alcohols and pyridines
- Both compounds are acidic in nature

According to the polyphenol theory, humic substances are formed from polyphenols. There are two variations of this theory. One assumes that polyphenols are formed from lignin, while the other assumes that polyphenols are formed from non-lignin sources (e.g., cellulose). The formation of humic substances from polyphenols in both these variations is the same. Polyphenols are oxidized to quinones by polyphenol oxidase enzymes. Quinones are then polymerized in the presence or absence of amino compounds to form fulvic acids, humic acids and humins (Abaecherli and Popa, 2015; Zanella *et al.*, 2018).

The sugar-amine theory postulates that the microbial degradation of organic residues produces reducing sugars and amino acids. These compounds undergo non-enzymatic reactions to form brown coloured nitrogenous compounds. The reaction proceeds via the addition of the amine to the aldehyde group of the reducing sugar, forming N-substituted glycosyl amine. This compound is unstable and undergoes Amadori rearrangement to form ketose amines (Souza *et al.*, 2020). The ketose amine reacts further to form new compounds through fragmentation (acetol, diacetyl) and dehydration (reductones, furfurals). These compounds undergo polymerisation reactions with amino compounds to form brown coloured substances. However, this reaction proceeds slowly under normal soil conditions, which is the main objection against this theory (Thevenot *et al*., 2010; Abaecherli and Popa, 2015).

2.4.1 Role of humic substances in the soil

The importance of SOM in maintaining agricultural productivity is indisputable (Schmidt *et al*., 2011; Sen *et al.*, 2020). Increasing the amount of SOM in agricultural systems is important both for sequestering atmospheric carbon dioxide and for improving soil productivity (Lal, 2006). The crop production potential of soil is related to its SOM content because SOM is crucial in maintaining the physical, chemical, and biological characteristics of the soil that are essential for fertility. Thus, maintaining SOM is crucial in ensuring long-term soil productivity (Fageria, 2012; Rickson *et al.*, 2015). When SOM is depleted, the plant biomass yield decreases due to a degraded soil structure, and the depletion of nutrients that were contained in SOM. As a result, the biota and biodiversity in the soil ecosystem declines (Pimentel and Burgess, 2013). Humic substances (humic acids, fulvic acids, and humins) make up 65% to 70% of humus (Gulser *et al.*, 2010). They perform numerous functions in the soil because of their structural characteristics, as summarised below (Chen, 2006; Krull *et al.*, 2004);

Physical functions

- Humus improves the bonding between mineral particles and facilitates the formation of stable soil aggregates. This improves water infiltration, aeration, and root development. Stable soil aggregates are more resilient against erosion.
- Improves soil water holding capacity.
- Reduces surface crusting, which enhances seed germination.

Chemical functions

- The cation exchange capacity (ability to adsorb plant nutrients) of humus (100 – 550 $cmol_c$/kg) is higher than that of clay (2 – 180 $cmol_c$ per kg). Soil with more humus can thus adsorb more cations (eg. Ca^{2+}, Mg^{2+}, K^+, NH_4^+) for plant uptake.
- Improves the pH buffer capacity of the soil.
- Sequestration of atmospheric carbon dioxide.
- Humus forms stable complexes with plant micronutrients such as iron, copper, zinc, and nickel. These chelates are more water soluble and available for plant uptake.
- Phosphorus deficiencies may occur in the soil because soluble inorganic phosphorus is fixed by aluminium and iron in acidic soils, and by calcium in alkaline soils. Soil organic matter improves phosphorus availability by forming complexes with these cations.

Biological functions

- Serves as a source of energy for soil microorganisms. Soils with higher humus contents have higher microbial populations and diversity.
- Enhances soil microbial diversity, which assists in disease suppression.
- The decomposition of SOM releases essential plant macro and micronutrients.
- Enhances the symbiosis between plants roots and fungi, and thus improves nutrient uptake.

2.4.2 Role of humic substances in plant metabolism

Humic substances also affect plant growth directly. These effects have been observed as changes in plant metabolism following the uptake of humic substances (Abaecherli and Popa, 2005). Humic substances cause various changes in cytoplasmic components of plant cells and cell membranes, affecting biochemical processes such as photosynthesis, respiration, nucleic acid synthesis and nutrient uptake (Nardi *et al.*, 2002; Ouni *et al*., 2014). The following physical effects have been reported in literature (Paksoy *et al.*, 2010; Trevisan *et al.*, 2010; Ouni *et al.*, 2014);

- Stimulates seed germination and plant hormone activity
- Improves plant growth
- Improves nutrient uptake
- Improves protein synthesis

2.5 Lignin as a raw material for nitrogen releasing soil conditioning materials through ammoxidation

2.5.1 Introduction

Lignin is a precursor of humus, and is a sustainable, renewable raw material that is abundantly available as a by-product of the pulp and paper industry, as discussed in section 2.3. Furthermore, quinone structures derived from lignin inhibit nitrification in the soil (Huang *et al.*, 2003; Abaecherli and Popa, 2005). This has allowed this biopolymer to be utilized in the manufacture of numerous agricultural products such as fertilizers, soil conditioners, seed coatings, slow-release pesticides and heavy metal sequestration products (Abaecherli and Popa, 2005; Chen *et al*., 2020b).

However, technical lignins are chemically different from natural lignins, and differ according to their extraction methods. Secondly, technical lignins are obtained from spent pulping liquor (black liquor) which also contains polysaccharides, extractives, and inorganics (ash). Thus, the direct application of black liquor in the soil may result in undesirable effects (Fischer and Schiene, 2002) . Black liquor has a high biological oxygen demand, chemical oxygen demand, and solids content, which can make it environmentally harmful (Grover *et al.*, 1999). It is thus necessary to isolate and purify technical lignins before further utilization. Thirdly, lignins have a low macronutrient content, and significantly high C/N ratio for use as soil amendments (Fischer and Schiene, 2002; Ragab, 2008).

Ammoxidation is process technology that uses an oxidant (usually pure oxygen or air) and ammonia to chemically bind nitrogen with lignin. The products of this process are called "N-lignins." Binding nitrogen to lignin decreases the C/N ratio, making lignin suitable for use as a soil amendment. Nitrogen is incorporated in different functional groups, which mineralize at different rates in the soil. Thus, only a fraction of the nitrogen is immediately plant-available, while the remainder is released slowly over time as the material degrades. The nitrogen functional groups have been classified as (in the order of decreasing hydrolyzability) (Fischer and Schiene, 2002);

1. Ammonium (NH_4^+ - N),
2. Amide (amide – N), and
3. Strong organically bound (sob – N).

2.5.2 Lignin ammoxidation: State of the art

Earlier studies in lignin ammoxidation focused on the production of organo-mineral fertilizers containing up to 19% nitrogen (C/N < 2.5). High oxygen pressures (20 – 120 bar) and high ammonia concentrations (4.3% to 15.5% NH_3 / reaction mixture) were used to synthesize these materials (Phillips *et al.*, 1934; Kobe *et al.*, 1936; Fischer and Schiene, 2002). Recently, the focus has shifted towards the synthesis of N-lignins that have a C/N ratio similar to that of humified SOM (10 – 15) (Ghorbani *et al*., 2017). In this regard, a process technology that uses ambient pressure, lower temperatures (70 - 80 ^oC) and lower ammonia concentrations (< 8% NH_3/ reaction mixture) was developed. Ambient pressure N-lignins contain lower nitrogen content (~5%), and a higher fraction of ammoniac nitrogen (9.8 - 41.4% N/N_T) in contrast to high pressure N-lignins (13.1% - 28.9% N/N_T). Furthermore, ambient pressure ammoxidation uses longer reaction times (~4 hours) in contrast to high pressure ammoxidation (~1.5 hours). However, low pressure ammoxidation exerts a greater oxidative attack on aromatic units, reduces operating costs and offers a safer operation (Fischer and Schiene, 2002).

The biomass origin, severity of the pulping process and the molecular weight distribution (discussed in section 2.3) affect lignin reactivity and nitrogen incorporation during ammoxidation. Meier *et al.* (1994) ammoxidized different lignin types at moderate conditions (130 ^oC, 10 bar oxygen pressure) and reported that the lignin's reactivity to ammoxidation increased in the following order: lignosulphonate > kraft lignin > soda lignin. The high reactivity of a spruce sodium lignosulphonate obtained was attributed to its ease of oxidation. Kinetic studies have revealed that oxidation is the rate controlling step for nitrogen enrichment. Capanema *et al.* (2001a) reported that the reaction rate is first order with respect to oxygen pressure and 0.5 order with respect to ammonia concentration. It was also shown that nitrogen enrichment correlates linearly with oxygen consumption, carbon dioxide formation, and the degradation of methoxyl groups (Capanema *et al.*, 2001a; Capanema *et al.*, 2001b; Capanema *et al.*, 2002, Capanema *et al.*, 2006).

The reaction mechanisms reveal that nitrogen is incorporated onto the oxygen-rich functional groups of lignin structures (Fischer and Schiene, 2002; Ragab, 2008). It has been shown that hardwood lignins can incorporate more nitrogen because of their higher methoxyl group content and less condensed structure (Fischer and Schiene, 2002). In some cases, lignin substrates have been oxidised prior to ammoxidation (i.e. pre-oxidised) to improve nitrogen incorporation. For example, Tyhoda (2008) reported that the nitrogen content of N-lignins obtained from lignocellulosic residue increased from 2.64% to 3.08% when the material was pre-oxidised with 1.5% H_2O_2. Similarly, Meier *et al.* (1994) found that the ammoniation of pre-oxidized lignin yielded N-lignins with double the amount of nitrogen incorporated into lignins without pre-oxidation. Pre-oxidation changes the functional group composition of lignin, resulting in an increase in oxygen-rich functional groups onto which nitrogen is bound (Meier *et al*., 1994; Tyhoda, 2008; Jeong *et al*., 2018).

The reaction temperature and time both have a positive effect on the nitrogen enrichment and affect the distribution of the nitrogen functional groups. Fischer and Schiene (2002) reported that increasing the reaction temperature from 105 ^oC to 180 ^oC resulted in a proportional increase in N_{tot} from 11.5% to 18.7%. Meanwhile, NH_4^+-N decreased, sob-N increased, and amide-N increased slightly (up to a temperature of 120 ^oC). It has thus been postulated that amide-N is formed as a result of ring cleavage reactions (Fischer and Schiene, 2002; Liebner *et al.*, 2016). Capanema *et al.* (2001a) reported that ammoxidation occurs over two-time phases. In both these phases, N_{tot} and the degradation of methoxyl groups increase linearly with the reaction time. The first reaction phase was estimated to have a reaction rate that is 2.3 times faster than the second phase. Both reaction phases occurred via similar reaction mechanisms, without a notable change in the nitrogen functional groups in the second phase (Capanema *et al*., 2001a).

2.5.3 Reaction mechanisms

Lignins undergo various structural modifications during ammoxidation. Aromatic structures, ether bonds, and alcohol groups are degraded, while C=O, C-N and C-N-H bonds are formed (Meier *et al.*, 1994; Fischer and Schiene, 2002; Ramírez *et al*., 2007). The degradation of methoxyl groups and desulphonation have been identified as the characteristic steps in the ammoxidation of spent sulphite liquor (Fischer and Schiene, 2002). The extent of these modifications increases with increasing nitrogen incorporation (Ramírez *et al*., 2007). The reaction mechanism is dependent on the phenolic moiety (phenolic or etherified) of the lignin structure.

Ammoxidation of phenolic structures

The reaction begins with a one electron oxidation of the phenolate structure. This is the rate determining step. The resulting phenoxy radical is attacked by a hydroxyl radical forming a hemiacetal, which undergoes demethoxylation and an ortho-quinone is formed. This increases the electron density on the alpha carbon atom, hindering an ammonia nucleophilic attack. Nitrogen is incorporated onto the orthoquinone via a 1, 2-addition of ammonia, forming quinone imines. These quinone imines can react with other quinones to form nitrogen containing polymers. The orthoquinone hemiacetal may also undergo oxidative ring cleavage reactions forming dicarboxylic acids, into which nitrogen may be incorporated in the form of ammonium salts and amide groups (Fischer and Schiene, 2002; Ragab, 2008; Tyhoda, 2008), as shown in Figure 13.

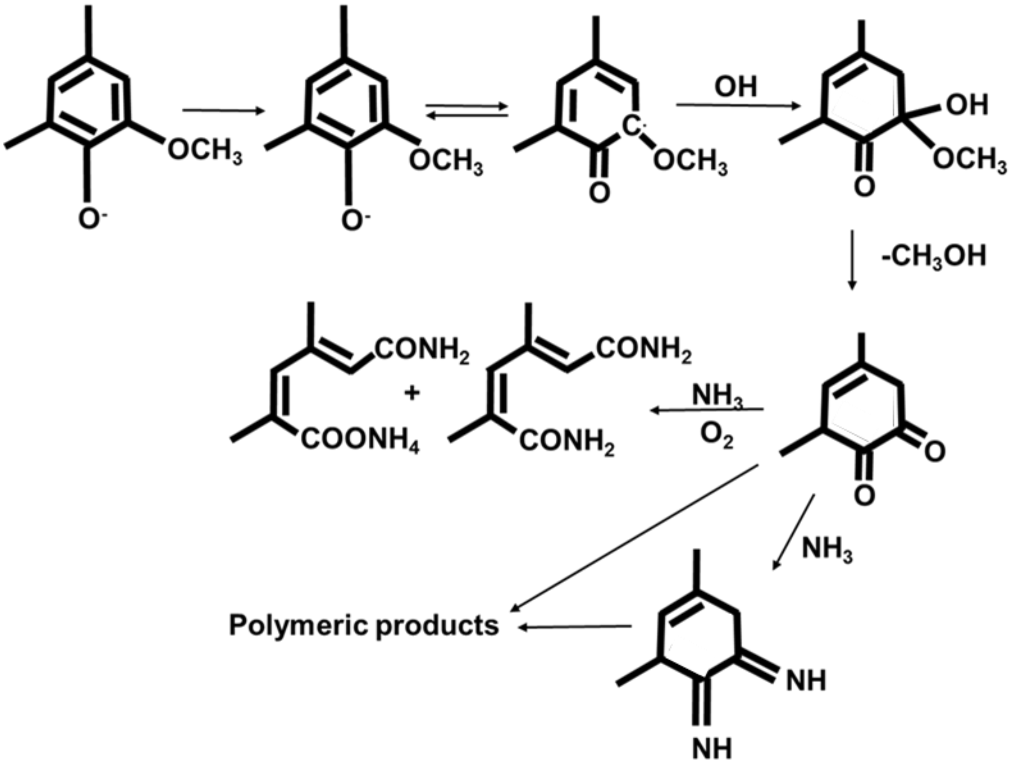


Figure 13 : Degradation of phenolic structures during lignin ammoxidation (Fischer and Schiene, 2002)

Ammoxidation of non-phenolic structures

The ammoxidation of non-phenolic structures begins with side chain degradation (cleavage of β – aryl and α – β bonds); this forms phenolic and non-phenolic lignin fragments. The phenolic fragments undergo ammoxidation as described already (section 2.5.3). Nitrogen is incorporated onto the non-phenolic fragments via a nucleophilic attack on the α-carbon atom, forming nitrile and amide groups, while the etherified structure is not degraded. Urea is formed from the carbon dioxide released during the degradation (Fischer and Schiene, 2002; Ragab, 2008), as shown in Figure 14.

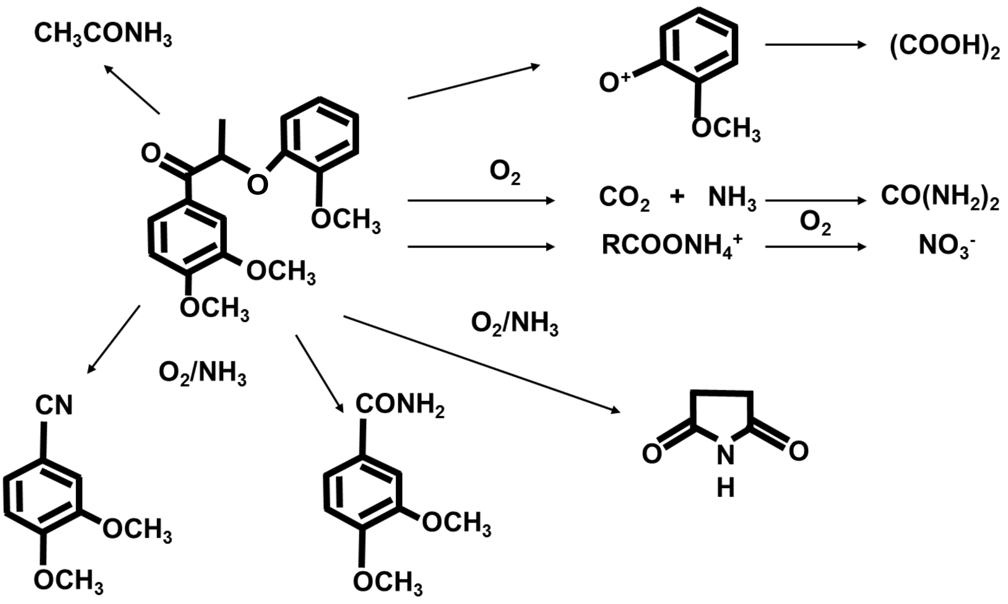


Figure 14 : Ammoxidation of non-phenolic lignin structures (Fischer and Schiene, 2002)

2.5.4 Lignin ammoxidation vs natural humus formation

The chemical reactions that occur during lignin ammoxidation are similar to those that occur during natural lignin degradation (section 2.3.4 - 2.3.5). Both processes are accompanied by a reduction of the mass fractions of carbon on the lignin, while nitrogen and oxygen contents increase. The content of methoxyl groups decrease, while carbonyl groups and carboxylic acid groups increase. However, ammoxidation utilises harsher process conditions and thus requires shorter reaction times compared to natural humification. In fact, lignin ammoxidation can be described as an accelerated natural humification (Kamble and Bhattacharyulu, 2015). Table 5 is a comparison of the properties on N-lignins and natural humus, most of which are similar. However, N-lignins have a lower CEC compared to natural humus because of a lower quantity of carboxylic acid groups in their structure (Fischer and Schiene, 2002).

Table 5 : Comparison of natural humus and N-lignins (Fischer and Schiene, 2002)

	Natural humus	**N-lignin**
C%	41 - 62	49 – 59
N%	1 - 5	3 - 5
NH_4-N	10 - 25	10 - 41
Amide-N	21 - 45	14 - 21
Sob-N	~50	44 – 73
Methoxyl group %	0.9 – 1.8	10 - 12
CEC	180 - 500	140 - 180
Molecular weight (g/mol)	10^4 – 2 x 10^5	>10^4

2.5.5 Novihum^{TM}

Lignite (brown coal) is chemically similar to lignin, contains more than 80% humic substances, and is an abundant resource in Germany. This had led to the production artificial humic substances, trade named Novihum^{TM}, through the ammoxidation of lignite. Nitrogen is incorporated into brown coal via the same reaction mechanisms that occur during lignin ammoxidation (Karapanagioti *et al*., 2010; Schillem *et al*., 2019). A Novihum^{TM} production plant started operation in 2016 in Dortmund, Germany. More than 400 successful plant trials have been conducted with Novihum^{TM} in different crops and soil types. The recommended application rate is 750-1 500 kg/ha, once in 3 to 5 years. Some of the benefits of Novihum^{TM} include: Increased crop yields and improved crop quality, improved soil water retention capacity, improved CEC, and faster growth rates (Tyhoda, 2008; Karapanagioti *et al.*, 2010). The chemical properties of Novihum^{TM} are shown in Table 6.

Table 6 : Chemical properties of Novihum^{TM}

CEC	140 – 180 mmol$_c$/100g
Density	0.8 kg/L
Carbon	55.2%
Nitrogen	4.7%
Ammoniac nitrogen	1.5%
Ash	5.1%

However, brown coal is a non-renewable resource. On the other hand, technical lignins are an abundant renewable resource, whose global production is expected to increase with the increase in pulp and paper production (Beckham, 2018). The industrial production of N-lignins may be achieved through an extension of existing pulp and paper production plants (Tyhoda, 2008). The industrial production of N-lignins could thus be advantageous compared to the industrial production of coal based humic materials.

2.5.6 Performance of N-lignins in pot trials

Numerous plant trials aimed at assessing the suitability of N-lignins for use as slow-release nitrogen fertilizers and soil conditioning materials have been conducted (Meier *et al*., 1994; Ramírez *et al*., 1997, 2001, 2007; Tyhoda, 2008). In this regard, the following criteria have been used;

- C/N less than 20
- Nitrogen content: 1% to 5%

N-lignins not only act as slow-release nitrogen fertilizers but also improve water holding capacity, CEC (Fischer and Schiene, 2002), adsorb noxious substances, bind carbon dioxide as carbamates in the soil (Liebner *et al.,* 2011,2016), reduce nitrate leaching (Ramírez *et al.*, 1997), and improve soil microbial activity (Fischer and Schiene, 2002). These properties make N-lignins superior to conventional nitrogen fertilizers and opens opportunities for their use in soil bioremediation applications (Abaecherli and Popa, 2005). High nitrogen contents in N-lignins would require lower N-lignin application rates for the targeted nitrogen application, and thus forfeit their soil conditioning benefits (Tyhoda, 2008).

Conventional fertilizers have a high water-solubility and make nitrogen immediately available for plant uptake and susceptible to various loss mechanisms. In contrast, N-lignins release nitrogen over an extended period. For example, the application of 1 385 kg/ha N-lignin increased sorghum yield from 1 t/ha to 5 t/ha, compared to soil without any fertilizer application. However, this was only 82% of the yield obtained from ammonium sulphate fertilized sorghum (Meier *et al*., 1994; Ramírez *et al*., 2007). Ramírez-Can*o et al.*, (2001) cultivated sorghum over two harvesting periods using N-lignin and urea as the conventional reference. In the first harvest, the biomass yield of N-lignin fertilized sorghum was lower than urea fertilized sorghum. However, in the second harvest (without a second application of fertilizing material), the biomass yield of N-lignin fertilized sorghum was higher because the organically bound nitrogen in N-lignin became readily available, while most of the urea supplied nitrogen was utilized in the first cultivation period (Ramírez-Cano *et al*., 2001; Ramírez *et al*., 2007).

The ammoxidation of sugars, especially monomeric sugars, may lead to the formation of phytotoxic substances which inhibit plant growth (Fischer and Schiene, 2002; Tyhoda, 2008; Klinger *et al*., 2013). Ammoxidation at low temperatures (<100 ^{o}C) is not expected to cause a severe degradation of polysaccharides to monosaccharaides, which could form phytotoxic substances (Tyhoda, 2008). Klinger *et al*. (2013) showed that the ammoxidation of glucose and xylose at a concentration of 0.05 g/ml resulted in the formation of 163.5 mg/g-extract and 122.4 mg/g-extract at 100 ^{o}C, while cellulose and xylan produced 0.023 mg/g-extract and 0.328 mg/g-extract,respectively (the ammoxidation reaction mixtures were extracted using ethyl acetate (20 ml),dried over sodium sulphate and vacuum dried). Decreasing the temperature to 70 ^{o}C reduced the phytotoxic content obtained from glucose to 15.4 mg/g-extract. This suggests that a mild ammoxidation process (<100 ^{o}C) is least likely to produce phytotoxic substances if the raw material does not contain a significant amount of monomeric sugars.

2.6 Gap in literature

The available literature has explored the use of various technical lignin types as raw materials for N-lignin synthesis. However, most studies have focused on the use of high-pressure oxygen as the oxidant for ammoxidation. For example, Ramírez *et al.*, (2007) and Meier *et al.* (1994) conducted ammoxidation at 150 ^{o}C, using oxygen at 15 bar, while Capanema *et al.* (2001a) ammoxidized organosolv lignin at 130 ^{o}C, using oxygen at

12 bar. Even though the N-lignins obtained in these studies were found to be suitable organic amendments (C/N<20), it must be noted that pure oxygen poses a safety risk and can be an expensive option to implement on an industrial scale. It is thus necessary to assess the reactivity of lignins to ammoxidation using air as the oxidant.

Tyhoda (2008) showed that the ammoxidation of commercial lignins using air at ambient pressure also produced N-lignins with a suitable C/N. However, fewer South African industrial lignins have been considered as raw materials for N-lignin synthesis. Only calcium lignosulphonate obtained from LignoTech and an autohydrolysis bagasse lignin from Illovo Sugar Ltd have been ammoxidized using air previously (Tyhoda, 2008) in South Africa. The present study sought to investigate the ammoxidation of South African lignins that have not been investigated previously (sodium lignosulphonate, soda lignin, kraft lignin) using air at 10 barg as the oxidant. The sodium lignosulphonate was of particular interest because it was obtained from a semi-chemical pulping process, and originated from a mixture of hardwood and softwood, making it unique compared to the lignins studied previously.

2.7 Research questions and Objectives

There is a gap in the literature regarding the use of South African technical lignins as raw materials for the synthesis of N-lignins, especially NSSC lignosulfonate. The following research questions were formulated for this study, to fill this gap.

2.7.1 Research questions

1. Can the selected raw lignins be ammoxidized using air at 10 barg to produce N-lignins with suitable C/N for use as soil organic amendments?
2. How does the application of N-lignins synthesized from the selected lignins at the selected conditions affect the growth of plants?

2.7.2 Research objectives

The following is a list of objectives formulated for the study. Objectives 1, 2 and 3 focused on research question 1, while objective 4 focused on research question 2.

1. Characterization of the raw lignins

The raw lignins were characterized to determine their elemental and structural composition which provided insight into their reactivity to ammoxidation. Raw material characterization included; elemental composition, ash content, total sugar content and functional group composition using FTIR.

2. Ammoxidation

The raw lignins were pre-oxidized and ammoxidized in a laboratory scale setup according to methods reported previously, but employing unique reaction conditions: 10 barg air pressure, 80 ^{o}C, 7% NH_3 mixture and 10% lignin mixture. It has been shown in previous studies that the extent of nitrogen incorporation during ammoxidation increases with an increase in temperature and oxidant pressure. However, significant amounts of N-heterocyclic compounds are formed when ammoxidation is carried out at temperatures beyond 100 ^{o}C. The selected reactions conditions were thus aimed at imparting the greatest extent of modification on the raw materials without an excessive formation of N-heterocylic compounds nor employing extreme operating conditions.

3. Characterization of the N-lignin products

The synthesized N-lignins were characterized to determine their C/N, total nitrogen content, ammonium bound nitrogen, pH, salinity, and water retention capacity. The functional group changes that occurred on each raw material were also analyzed using FTIR.

4. Plant toxicity and nitrogen uptake assessment

The synthesized N-lignins were tested in a plant trial using white mustard seedlings (*Sinapis Alba*), to assess their suitability for use as soil organic amendments (plant toxicity and nitrogen uptake). The height, dry mass yield, and total nitrogen content of the seedlings were analyzed after a four-week cultivation period.

3 AMMOXIDATION OF SOUTH AFRICAN TECHNICAL LIGNINS

3.1 Experimental approach

Spent pulping liquor samples were collected from three different mills that use the kraft, soda and NSSC processes, respectively. Soda and kraft lignins were precipitated from their respective pulping liquors and purified before use, while sodium lignosulphonate was used directly in its liquor form after filtering out any foreign matter that may have been present. The moisture content, ash content, elemental composition and FTIR spectra of the three materials were determined. The characterized materials were then ammoxidized to synthesize N-lignins, which were analyzed for elemental composition, water holding capacity, pH, salinity, functional group composition through FTIR, and nitrogen functional groups (mineral or organic). Lastly, the N-lignins with C/N < 20 were tested in pot trials using white mustard seedlings (*Sinapis alba*) over a growth period of 4 weeks. The growth rate, fresh mass yield, and nitrogen accumulation in seedlings was assessed. The experimental approach is summarized in Figure 15.

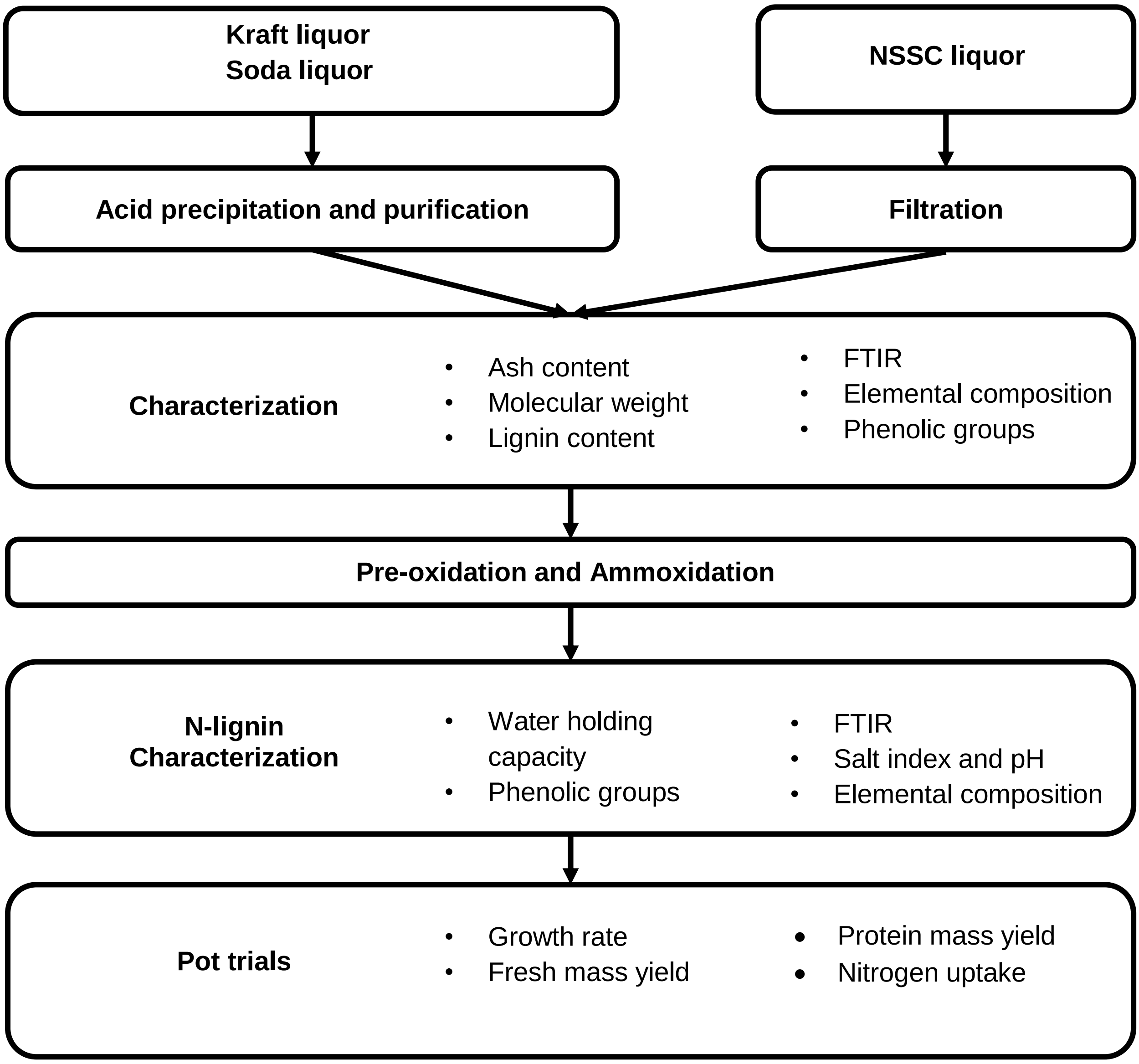


Figure 15 : Experimental approach

Table 7 shows the biomass origin, pulping process and samples codes used for the starting materials. These sample codes were used throughout this report. The prefix “N-” was used for samples that were ammoxidized only, while “N-$O_{1.5}$-” and “N-O_5-” were used for samples that were ammoxidized after a pre-oxidation with 1.5% H_2O_2 or 5% H_2O_2, respectively.

Table 7 : Lignin types used as raw material

Mill	Pulping process	Biomass origin	Sample code
Mpact, Piet Retief	NSSC	Hardwood (Eucalyptus) – 55% and softwood (Pinus) – 45%	SLS
Mondi, Richards Bay	Kraft	Hardwood (Eucalyptus)	KRT
Sappi, Stanger	Soda	Bagasse (Saccharum)	SOD

3.2 Lignin precipitation and purification

Sodium lignosulphonate liquor was filtered using type 1 Whatman filter paper and used without any further purification. Kraft and soda lignins were precipitated at pH 2 over a 24-hour period by adding 40 - 50 ml of 98% sulphuric acid per litre of liquor. The acidified mixture was then centrifuged at 7 000 rpm for 10 minutes to recover the lignin. The recovered lignin was washed with reverse osmosis water and centrifuged at 7 000 rpm for 10 minutes. The washing step was repeated twice. The washed lignin was then air-dried in a fume hood. The dried lignin was milled using a Retsch ZM 200 ring and puck mill, using a sieve of 0.5 mm aperture size, at 6 000 rpm. These materials were further purified by soaking, over a 24-hour period, in a 0.5 M sulphuric acid solution in a ratio of 20 L of sulphuric acid solution per 100 g lignin. The suspension was subsequently vacuum filtered using type 1 Whatman filter paper, washed with reverse osmosis water, and air-dried. The air-dried lignin was then used for pre-oxidation and ammoxidation.

3.3 Characterization of the starting materials

Moisture content

The moisture contents of the raw materials were determined according to the method reported by the Technical Association of the Pulp and Paper Industry (TAPPI) T2264 cm-07. A sample (~ 2 g) was weighed in a crucible and oven-dried at 105 °C over 24 hours. After this, the sample was cooled in a desiccator, weighed and oven dried again for one hour. This process was repeated until the sample reached a constant mass. The moisture content was then calculated according to Equation 1, and the solids content was determined by difference.

$$\text{Moisture (\%)} = \frac{\text{Sample mass (g)} - \text{Sample oven dry mass (g)}}{\text{Sample mass (g)}} \times 100$$ **Equation 1**

Ash content

The ash content was determined according to NREL/TP-510-42622. A crucible was heated to 575 °C in a muffle furnace for four hours, cooled in a desiccator for two hours, and weighed. The crucible was then used to heat an oven dried sample (~1.5 g) to 575 °C in a muffle furnace for 24 hours. The sample and crucible were then cooled for 2 hours in a desiccator, weighed, and kept in the muffle furnace for 4 hours. This process was repeated until the sample reached a constant mass. The ash content was then calculated in triplicate according to Equation 2 below;

$$\text{Ash (\%)} = \frac{\text{Mass of sample and crucible} - \text{Mass of crucible}}{\text{Mass of oven dry sample}} \times 100$$ **Equation 2**

Sodium lignosulphonate content in NSSC liquor

The sodium lignosulphonate content in NSSC liquor was determined according to the method reported by Lin (1992). NSSC liquor (~ 2 ml) was dried in the oven at 110 °C until constant mass. A sample (0.25 g ± 0.001 g) of this dried material was weighed in a 50 ml beaker, 40 ml deionized water was added, and stirred until the solid material was completely solubilized. An acetic acid solution (50 wt. %) was added dropwise while stirring, until the pH was between pH 5 and pH 5.5. The solution was then transferred into a 2 L volumetric flask, deionized water was added to the mark, and the flask was shaken to homogenize the solution. The absorbance of this solution was read from a photo-spectrometer at 280 nm, and the lignosulphonate content was calculated using Equation 3 below;

$$\text{NaLS (\% dry matter)} = \frac{\text{Absorbance at 280 nm} \times \text{V (ml)}}{\varepsilon \times \text{m (g)} \times \text{Pathlength}} \text{ x } 100$$ **Equation 3**

Where;

V : Volume of sample solution (2 L)

ε : Absorptivity of biomass at 280 nm

m : Mass of oven dry sample

Path length : Length of the photo-spectrometer cell, 1 cm

Elemental composition

The elemental compositions of both the raw and ammoxidized lignins were determined according to ASTM D4239 (sulphur) and ASTM D5373 (carbon, hydrogen, and nitrogen) methods at the Central Analytical Facilities (CAF), Stellenbosch University. Each sample was introduced into a combustion chamber set at 1 050 - 1 150 ^{o}C with tungsten trioxide (~5 mg) and dosed with oxygen gas at 2 bar. The CHNS composition was determined from the water, carbon dioxide, sulphur oxides, and nitrogen oxides that were released from the combustion using a Vario El Cube Elemental analyzer.

Total sugar

The total sugar contents in the starting materials were determined using the phenol sulphuric assay, AOAC (Association of Analytical Chemists) method 988.12, as adapted from Rover *et al.* (2013). Each sample was dissolved in reverse osmosis water (sodium lignosulphonate) or a 0.05 M sodium hydroxide solution (KRT and SOD) to a concentration of 150 - 350 mg/ℓ. From this solution, 1 ml was pipetted into a 10 ml vial, followed by 1 ml of a 5% phenol solution. The bottles were swirled, and 5 ml of 98% sulphuric acid was added into the mixture. The bottles were swirled, allowed to stand for 10 minutes, and placed in a water bath set at 30 ^{o}C for 20 minutes. The absorbance of each sample mixture was then read from a UV-Vis spectrophotometer at 480 nm. A calibration curve (Figure 16) was constructed using xylose solutions of different concentrations, ranging from 0 to 60 mg/L. The total sugar content of each sample was back calculated from the calibration curve using the sample solution absorbances.

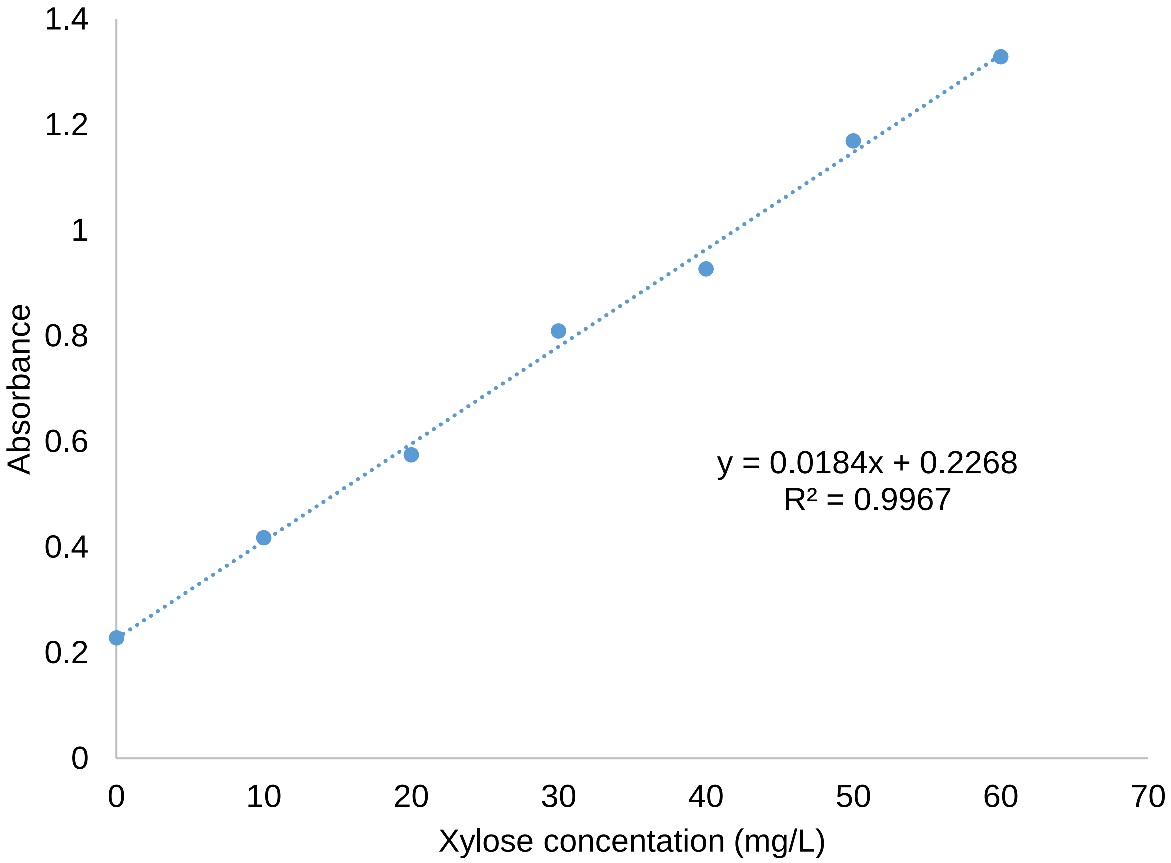


Figure 16 : Total sugar - calibration curve

Molecular weight

The weight average (M_w) molecular weights, number average (M_n) molecular weights and polydispersity index (M_w/M_n) of lignin samples were determined through size exclusion chromatography, using an Agilent 1260 infinity Quaternary LC. The unit comprised of two Aqueous Agilent polargel-M (300 x 7.5 mm) columns, with a guard column, set at a temperature of 60 ^{o}C, and a variable wavelength detector set a wavelength of 254 nm and a temperature of 30 ^{o}C. The solvent was a mixture of dimethylsulfoxide and water (9:1, v/v), containing lithium bromide, which was dissolved to a concentration of 0.05 M. Each sample was dissolved in this solvent to make a concentration of 1 mg/ml, filtered using a 0.45 µm syringe filter, and kept in HPLC vials. The solvent was also used as the eluent for the analysis and was supplied at a flowrate of 0.5 ml/min and a pressure of 68 bar. The analysis was performed for a period of 45 minutes after injecting 40 µL of the sample solution. Polysaccharide standards (pullulan) were used for the calibration of the unit for molar masses ranging from 180 g/mol to 708 000 g/mol. This method was adapted from Du Toit (2021).

Phenolic hydroxyl group content

The content of phenolic hydroxyl groups was determined according to the method reported by Areskogh *et al.*, (2010). Sample solutions (0.5 g/L) were made using reverse osmosis water for sodium lignosulphonate, and 0.5 M sodium hydroxide solution for kraft and soda lignin. Reverse osmosis water (30 ml) was added into a 250 ml Erlenmeyer flask, along with 3 ml of Folin-Ciocalteu (FC) reagent, and 1 ml of each sample solution. After ten minutes, 10 ml of a sodium carbonate solution (20%) was added into the Erlenmeyer flask. The mixture was then transferred into a 50 ml volumetric flask, and the volume was topped up to the mark using reverse osmosis water. The mixture was then stirred at 120 rpm inside an incubater for 2 hours, before measuring the absorbance at 760 nm using a photo spectrometer. Each measurement was conducted in duplicate. Known vanillin concentrations were made, and were used to construct a calibration curve – a plot of absorbance against concentration (Figure 17). Each vanillin solution was treated similarly to the sample solutions. The phenolic group contents of the sample solutions were determined by fitting their absorbance values to the calibration curve, noting that vanillin is a phenolic aldehyde. A new calibration curve was constructed for each FC reagent batch due to the sensitivity of this reagent to light.

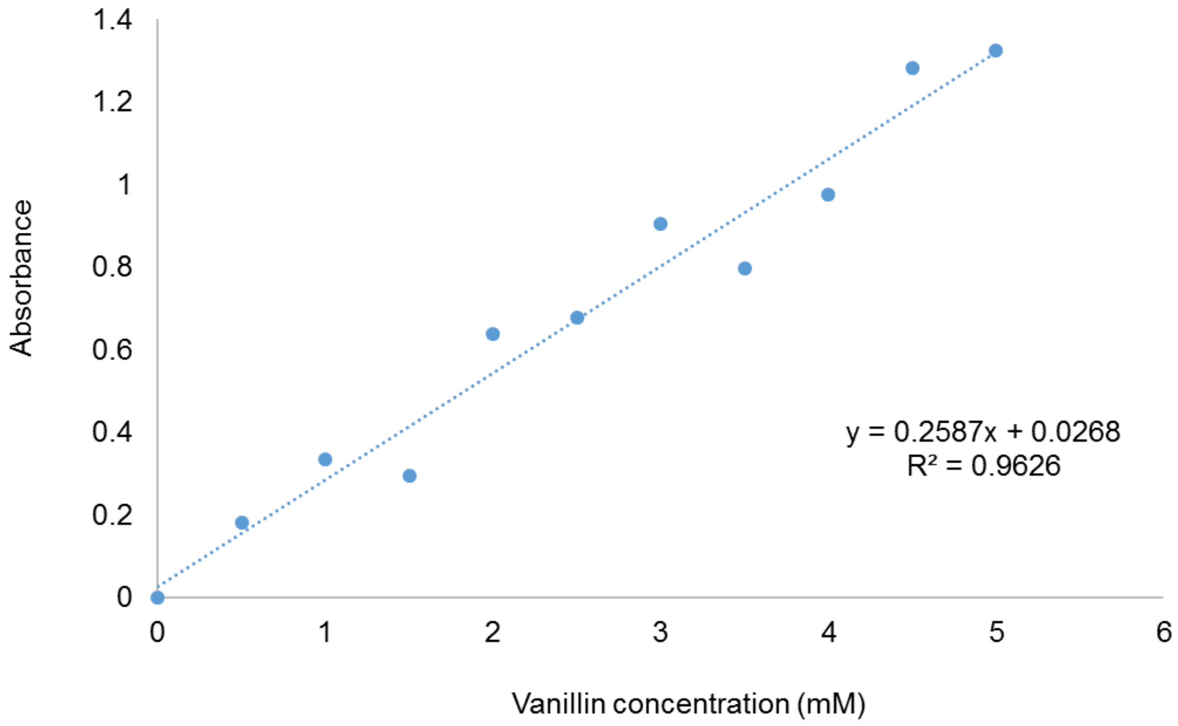


Figure 17 : Calibration curve for phenolic hydroxyl group determination

Macro and Micronutrient composition of raw lignins

The starting materials were analyzed for phosphorus, potassium, and micronutrient content (calcium, magnesium, sodium, iron, copper, zinc, manganese, boron, and aluminum) at the laboratories of the Department of Agriculture of the Western Cape at Elsenburg, according to the dry ashing method reported by the Agriculture Laboratory Association of South Africa (ALASA) (method 6.1.1) (Palic *et al.*, 1998). The method can be summarized as follows;

1. The samples were ashed at 460 °C overnight.
2. The ash was dissolved in concentrated hydrochloric acid, heated, and diluted with distilled water.
3. The sample mixture was then filtered using a 0.45 nm filter and analyzed using inductively coupled plasma mass spectrometry (ICP – MS).

Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) was used to investigate structural features that each raw material possesses, and the structural modifications that occurred during ammoxidation, using a Bruker Alpha II ATR-IR instrument. A background measurement was performed before each sample spectrum was recorded. Data was recorded for each sample in the range 4 000 - 400 cm^{-1} using a resolution of 4 cm^{-1}. Absorption peaks were identified, and the corresponding functional groups were identified based on literature (Faix, 1991; Ramírez *et al.*, 2007; Nandiyanto *et al.*, 2019).

3.4 Pre-oxidation and ammoxidation of lignins

Pre-oxidation and ammoxidation was carried out in a 1 L Parr reactor according to the method reported by Tyhoda (2008). However, a higher air pressure was used (10 barg instead of ambient pressure) for ammoxidation to impart a stronger oxidative attack on the raw materials and improve nitrogen incorporation. The reagent (hydrogen peroxide solution or ammonia solution, for pre-oxidation or ammoxidation, respectively) was diluted to the desired concentration inside a fume hood. Each sample was then loaded into the reactor, followed by the reagent solution. The reactor was then sealed, pressurized with air to 8 barg, and heated to the desired temperature (while stirring at 250 rpm) using an electrical heating jacket and a PID temperature controller (Parr 4848B controller). The heating time was approximately fifteen minutes. Upon completion of the reaction time, the heating jacket was removed, and a water bath was used to cool the reactor to 40 °C. A gas outlet valve was opened to release pressure in the system, and the reaction mixture was collected. The reactor setup is shown in

Figure **18**, and the reaction conditions used for the pre-oxidation and ammoxidation are shown in Table 8. Each ammoxidation (and pre-oxidation) run was conducted in duplicate.

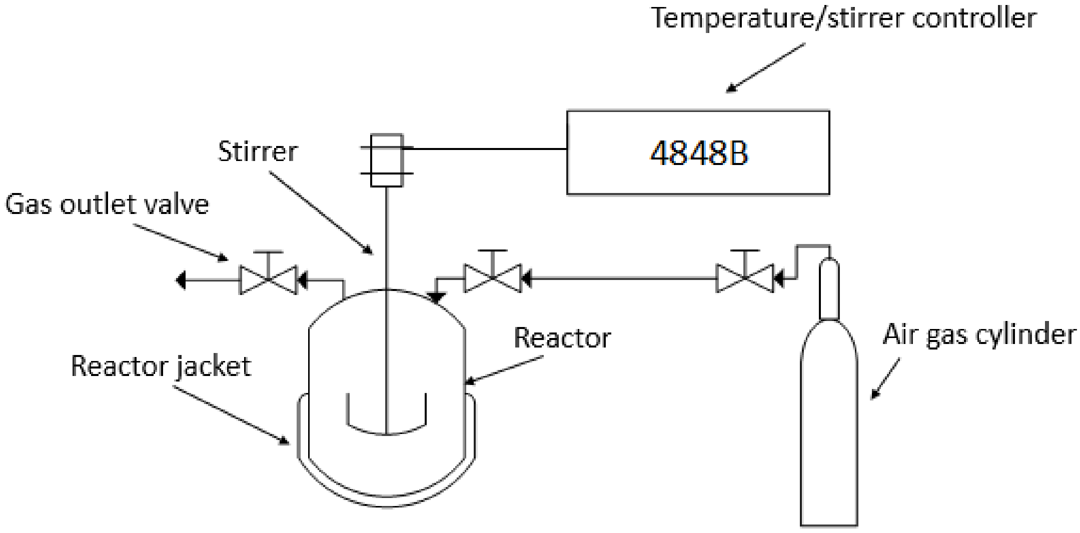


Figure 18: Schematic diagram of the reactor setup

Table 8 : Pre-oxidation and ammoxidation conditions

Parameter	**Value**
Temperature	40 ^oC
Oxidant	Hydrogen peroxide
Time	4 hours
H_2O_2 mass % mixture	1.5 and 5
Lignin mass % mixture	9
Stirrer speed	250 rpm
Ammoxidation conditions	
Temperature	80 ^oC
Oxidant	Air
Pressure	10 barg
Time	4 hours
Ammonia % mixture	7
Lignin % mixture	10
Stirrer speed	250 rpm

3.5 Spray drying

The reaction mixtures obtained from the pre-oxidation and ammoxidation reactions were dried using a Büchi Mini Spray dryer B-290 to obtain the N-lignins, using conditions shown in Table 9. In this unit, the feed mixture is sprayed into fine droplets and mixed with a stream of hot air. This allows water to evaporate from the droplets, leaving the dry particles suspended in the air stream. The rapid evaporation of water keeps the particles at or below the spray dryer outlet temperature (68 ^oC). The dried particles are then separated from the exit gas using a cyclone. The outlet temperature (68 ^oC) was selected to avoid the thermal decomposition of nitrogen compounds in the products (Tyhoda, 2008). The unit is shown in Figure 19.

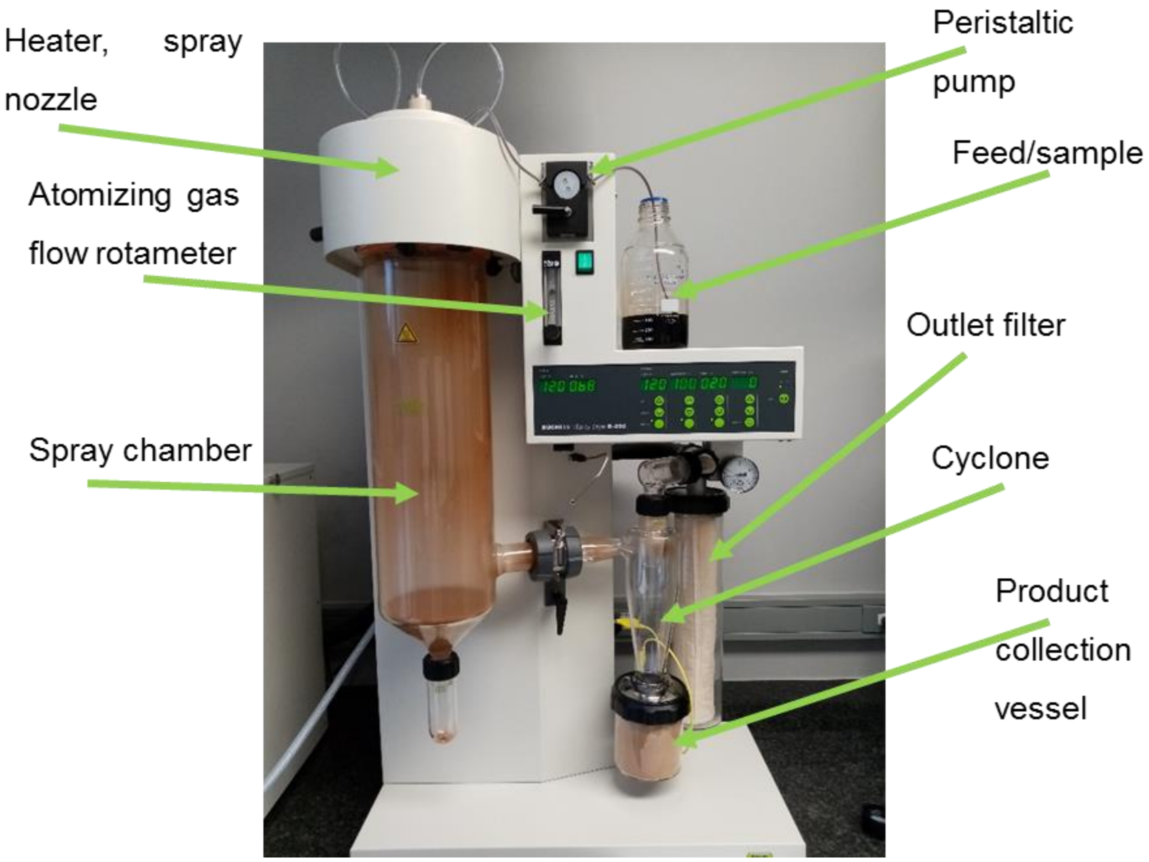


Figure 19: Büchi B-290 Spray dryer unit

Table 9: Spray drying conditions

Parameter	Value
Inlet gas temperature	120 ^{o}C
Outlet temperature	68 ^{o}C
Inlet air flowrate	40 mm (667 L/hour) *
Inlet air humidity, temperature	95%, -1 ^{o}C
Nozzle diameter	0.7 mm
Particle size range	2 – 25 µm

*At 20 ^{o}C and 1 atm

3.6 Characterization N-lignin products

Elemental composition and FTIR

The elemental composition and FTIR spectra of the N-lignins was determined as described in section 3.3 for the starting materials. The carbon to nitrogen ratio (C/N) was calculated using the total carbon content and the total nitrogen content from the elemental composition of these materials.

Ammonium bound nitrogen

Ammonium bound nitrogen was determined according to method 920.03 of the AOAC, also known as the magnesium oxide method (Helrich, 1990). A sample (150 mg ± 5 mg), carbonate free magnesium oxide (2 g ± 0.1 g), and 50 ml reverse osmosis water were added into a distillation flask. The mixture was distilled to release ammonium bound nitrogen in the form of ammonia gas. The released gas and water vapor were condensed and trapped in a sulphuric acid solution. This causes the ammonia gas to neutralize some of the acid solution. After distilling for fifteen minutes, the acid solution was back titrated using a sodium hydroxide solution. The amount of ammonium bound nitrogen in the sample was calculated as shown in Equation 4;

$$N_{NH4}\,(\%\ \text{sample}) = 2 \times 14.007 \times M_2 \times \left(V_2 - \frac{M1 \times V1}{2 \times M2}\right) \div W$$ **Equation 4**

Where; M_2 – Molarity of sulphuric acid solution

V_2 – Volume of sulphuric acid solution

M_1 – Molarity of sodium hydroxide solution

V_1 – Volume of sodium hydroxide used to back titrate the acid solution

W – Mass of sample (g)

Organically bound nitrogen

Organically bound nitrogen was determined as the difference between the total nitrogen content and the ammonium bound nitrogen content, using Equation 5;

$$\text{Organically bound N}\ (\%\ \text{sample}) = 100\ \% - (N_{NH4})$$ **Equation 5**

Electrical conductivity, pH, and Salt index

The pH, electrical conductivity (EC) and salt index (SI) were measured according to the methods reported by Li *et al*. (2021). Each sample (0.25 g ± 0.01 g) was mixed with 250 ml reverse osmosis water and stirred at 250 rpm using a magnetic stirrer for fifteen minutes. The pH and electrical conductivity were then measured using a Eutech pH 6+ pH meter and a Crison CM 35+ electrical conductivity meter, respectively. The salt index was calculated as shown in Equation 6;

$$\mathrm{SI} = \frac{\text{EC of sample solution}}{\text{EC of sodium nitrate solution}} \times 100\%$$ **Equation 6**

Water retention capacity

The water retention capacity was determined according to a method adapted from Wei *et al.*, (2019). One gram of dry sample was mixed with 100 g of oven dry sand in a 250 ml beaker. Reverse osmosis water (50 ml) was added into each beaker, weighed, and kept at ambient conditions. The beakers were weighed again ten days later. A control with sand only was used for comparison. Each measurement was completed in triplicate. The water retention capacity was then calculated as shown in Equation 7;

$$\text{Water retension capacity } (\%) = \frac{\mathrm{A-C}}{\mathrm{B-C}} \times 100\%$$ **Equation 7**

Where;

A: Mass of sand, water, and beaker on day 10

B: Mass of sand, water, and the beaker on day 0

C: Mass of sand, sample, and the beaker

Solubility

The water solubility of the N-lignins was measured according to a method adapted from Li *et al*. (2021). Each sample (1 g) was added into a 100 ml beaker and mixed with 50 ml of reverse osmosis water. The mixture was stirred at 300 rpm for 15 minutes and filtered through Whatman no.1 filter paper. The beaker, filter paper and undissolved solids were oven dried at 40 ^{0}C until constant mass. The solubility was calculated as shown in Equation 8;

$$\text{Solubility (g/l)} = \frac{\text{J}-\text{K}}{50\ \text{ml}} \times 1\ 000$$ **Equation 8**

Where;

J: Mass of beaker + mass of filter paper + mass of sample

K: Mass of dried filter paper, undissolved solids and beaker

4 Results and Discussion: Starting materials and N-lignins

4.1 Starting materials

Technical lignins from different pulping processes have unique structural properties, both due to the origin of the lignocelluloses from which it stems, and due to the structural modifications of lignins imparted by different pulping techniques. Moreover, KRT and SOD were purified while SLS was used without purification. It is thus important to characterize the technical lignins before ammoxidation as their structural properties affect the reactivity of each technical lignin to ammoxidation. Such a characterization step provides insights into the structure of lignin, and the level of reactivity that can be expected from each lignin type (Tyhoda, 2008; Beckham, 2018).

Elemental composition and ash content

KRT and SOD had carbon contents of 56.3% and 56.4%, respectively, while SLS had a carbon content of 28.3%, as shown in Table 10. These carbon contents are in agreement with findings from previous studies where these lignins were characterized (Naron *et al*., 2017; Govender *et al.*, 2020). Lignosulphonates typically contain lower carbon contents (Tyhoda, 2008). However, SLS contained a considerably lower carbon content compared to lignins that have been ammoxidized previously (Meier *et al*., 1994; Tyhoda, 2008). Lignosulfonates obtained from acid sulphite pulping have lower ash contents and higher carbon contents due to the pulping conditions used. For example, a calcium lignosulphonate (from softwood biomass) ammoxidized previously had a carbon content of 45.2% (Tyhoda, 2008), while a previously ammoxidized sodium lignosulphonate, of softwood origin (Spruce), had a carbon content of 57.9% (Meier *et al.*, 1994). Carbon is the substrate for organically bound nitrogen. According to Fischer and Schiene (2002), sulphite lignins still contained 39.5% - 40.9% carbon after ammoxidation.

Table 10 : Proximate analysis and elemental composition of starting materials

	C [%]	N [%]	H [%]	S [%]	C/N	Moisture [%]	Ash [%]
KRT	56.3	0.2	6.5	6.3	281.5	3.77	0.5
SOD	56.4	0.3	7.4	0.3	188.0	4.28	0.75
SLS	28.3	0.2	3.6	6.2	176.6	3.16	61.14

The starting lignins were also characterized by low nitrogen contents (0.2% to 0.3%) and high C/N ratios (177.6 to 281.5) (Table 10). As expected, these C/N ratios do not meet the criteria for use as soil organic amendments (C/N < 20) (Fischer and Schiene, 2002). The direct application of these materials in the soil would lead to nitrogen immobilization which is undesirable. It is necessary to enrich these lignins with nitrogen. N-lignins reported in literature typically had nitrogen contents in the range 3% to 5%, and C/N between 10 and 15 (Fischer and Schiene, 2002; Tyhoda, 2008; Ghorbani *et al.*, 2017). The C/N ratio and total nitrogen content of raw materials dictates the amount of nitrogen that must be incorporated to achieve C/N < 20 (Fischer and Schiene, 2002). Sucrolin^{TM} (commercial bagasse lignin) and Indulin^{TM} (commercial kraft lignin), ammoxidized previously, had higher nitrogen contents corresponding to C/N of 101.9 and 53.8 initially, respectively (Tyhoda, 2008). A greater extent of ammoxidation was thus required for the lignins used in this study to obtain C/N comparable to those achieved with commercial lignins in previous studies, due to higher initial C/N (Table 10).

The sulphur contained in these raw materials arises from the biomass, the chemicals used in the pulping process (except for SOD), and the sulphuric acid used in the precipitation and purification (Naron *et al.*, 2017). KRT (6.3%) and SLS (6.2%) had a higher sulphur content compared to SOD (0.3%) due to the pulping chemicals used (Table 10). The application of N-lignins obtained from these raw materials as soil organic amendments can thus avail an appreciable amount of sulphur in the soil (Kok *et al.*, 2021). Sulphur is an essential plant micronutrient that is necessary to produce amino acids that are involved in chlorophyll production, and for protein and vitamin synthesis in plants (Hofman and Cleemput, 2004).

KRT (0.5%) and SOD (0.75%) had lower ash contents compared to sodium lignosulphonate (61.14%) (Table 10). This is because KRT and SOD were precipitated and purified using sulphuric acid. SLS, due to the presence of sulphonic groups in its structure, has a high water-solubility and cannot be acid precipitated (Naron *et al.*, 2017). This starting material was thus used without any purification. Compared to lignosulphonates considered for ammoxidation in the literature, the ash content of SLS (61.14%) is considerably high compared to the calcium lignosulphonate (21.3%) ammoxidized by Tyhoda (2008), and the sodium lignosulphonate (23.2%) ammoxidized by Meier *et al*. (1994). Both scholars had ashed the materials at 525 ^{o}C according to the TAPPI method T 211 om-85. The higher ash content implies that SLS had a lower quantity of organic substrate to organically bind nitrogen compared to the lignins ammoxidized previously.

Generally, the alkaline lignins used in this study had chemical compositions that were in line with previous studies, except for higher initial C/N ratios. SLS had two distinct characteristics compared to the lignosulphonates considered for ammoxidation previously i.e., 1) high ash content (61.14%), and 2) a low carbon content (28.3%).

Macro and micronutrient composition

The ash content of raw lignins is of important consideration for ammoxidation because the inorganic elements in the ash may affect the rate of ammoxidation (Meier *et al.*, 1994). Furthermore, the presence of trace elements in the lignin materials may improve plant metabolic processes; P (energy storage and transfer), K (enzyme activation, transportation and assimilate transportation), Ca (cell wall stabilization), Mg, Fe and Zn (chlorophyll production), B (cell wall synthesis), and Cu (nitrogen uptake and hormone metabolism) (Hofman and Cleemput, 2004). These elements are therefore essential plant nutrients (Meier *et al.*, 1994; Fischer and Schiene, 2002; Tyhoda, 2008).

The macro and micronutrient content of the starting materials is shown in Table 11. Some of the trace elements in the raw lignins originated from the biomass before chemical pulping, while some of these elements may originate from the chemical reagents used in each pulping process, either from the reagents themselves or as impurities (Naron *et al.*, 2017).

Table 11: Macro and Micronutrient content of raw lignins (oven dry basis)

Element (mg/kg)	KRT	SLS	SOD
Phosphorus	0.01	0.01	0.01
Potassium	0.01	0.26	0.01
Manganese	0.49	32.79	0.56
Sodium	62	144 900	66
Iron	16.90	49.42	45.93
Copper	2.27	0.49	2.82
Zinc	0.57	2.10	1.31
Calcium	0.01	0.03	0.01
Boron	18.28	5.08	5.93
Aluminum	12	40	18
Magnesium	0.01	0.04	0.01

The high sodium content of SLS corresponded to its high ash content (61.14%) compared to KRT (0.5%) and SOD (0.75%) (Table 10, Table 11). Sodium originated from the chemicals (sodium hydroxide, sodium sulphite, sodium sulfide and sodium carbonate) used in the various pulping processes, as well as the biomass itself (Naron *et al.*, 2017). A high sodium content may lead to salinization in the soil, which may harm plants (Machado and Serralheiro, 2017). Residual sodium sulphite in the SLS will also compete with the oxidant, reducing the extent of ammoxidation on this raw material (Moodley *et al.*, 2012) . The dissolution of sodium in water results in an alkaline solution. It could thus be anticipated that the application of SLS in the soil could result in a higher pH compared to KRT and SOD. Changes in the soil pH may also affect nutrient uptake and plant growth (Li *et al.*, 2021).

The concentration of heavy metals is a limiting factor to the land application of organic materials. Even though some of these metals promote plant growth, over-application may be toxic (Li *et al.*, 2021). According to the South African fertilizer regulation, the maximum permitted levels of copper, zinc and boron in fertilizers are 750 mg/kg, 2 750 mg/kg and 80 mg/kg, respectively (Department of Agriculture, Forestry and Fisheries, 2012:50). All three raw materials constituted these elements at sufficiently low quantities to comply with this regulation, because these elements do not form part of the reagents used in the isolation of these lignins (Gellerstedt, 2009).

Phenolic hydroxyl groups, molecular weight, and total sugar content

The phenolic hydroxyl group content, molecular weights and total sugar contents of the starting materials used in this study are shown in Table 12 below. This structural information about each starting material was used to predict its reactivity to ammoxidation.

Table 12: Phenolic hydroxyl groups, molecular weights, total sugar of raw lignins

Lignin	Phenolic hydroxyl group content (mmol/g)	Molecular weights (g/mol)			Total sugar (wt. %)
		Mn	Mw	Mw/Mn	
KRT	6.05	221	2563	11.60	14.21
SOD	3.22	169	3936	23.29	23.39
SLS	0.37	1842	3532	1.92	7.74

The total sugar content obtained for these raw materials is within the range reported in literature (Sun *et al.*, 2003). SOD had a higher sugar content (23.39%) compared to KRT (14.21%) and SLS (7.74%) (Table 12). SOD was obtained from sugarcane bagasse, a non-wood biomass. Non-wood biomass has a high content of LCC, making it difficult to isolate the lignin from carbohydrates (cellulose and hemicellulose) compared to the isolation of lignin from woody biomass, resulting in a higher sugar content (Gellerstedt, 2009; Beckham, 2018). KRT was obtained from a harsher pulping process compared to SLS, resulting in more carbohydrate degradation, and a higher total sugar content in this raw material (Northey, 2001). Klinger *et al*. (2013) showed that the ammoxidation of monomeric sugars resulted in the formation of considerable amounts of phytotoxic substances (>120 mg/g-extract) at a temperature of 100 °C, but much lower quantities were obtained (<16 mg/g-extract) at 70 °C, at an ammoxidation concentration of

0.05 g sugar/ml. Thus, for the ammoxidation of lignins at 0.1 g/ml (present study) at 80 °C, with materials containing low total sugar contents (< 25%) (Table 12), the formation of toxic substaces was not deemed to be a concern.

KRT had a lower molecular weight (2 563 g/mol) compared to SOD (3 936 g/mol) and SLS (3 532 g/mol), as shown in Table 12. The molecular weights were within the range reported in literature (Naron *et al*., 2017; Du Toit ,2021). The molecular weight of lignin is affected by the severity of the pulping method. Harsher pulping methods lead to severe lignin fragmentation, resulting in lower molecular weights (Beckham, 2018). Furthermore, KRT originates from hardwood biomass only, unlike SOD and SLS. The abundance of G units in softwood and grass lignins makes their lignin structures more stable compared to hardwood lignin (Gellerstedt, 2009). G units often form stable C-C bonds with other monolignols at the C_5 position (which are difficult to cleave during delignification) and may re-polymerize during pulping (depending on the reaction conditions), resulting in higher molecular weight lignin fragments (Gellerstedt, 2009; Naron *et al.*, 2017).

KRT (6.05 mmol/g) also had a higher phenolic hydroxyl group content compared to SOD (3.22 mmol/g) and SLS (0.37 mmol/g) (Table 12). Similar findings were reported in literature (Naron *et al*., 2017; Antonino *et al*., 2021; Du Toit , 2021). The cleavage of inter-unit linkages during pulping results in the formation of low molecular weight lignin fragments, which contain more phenolic hydroxyl groups (Gellerstedt, 2009). Since most of the nitrogen incorporation during ammoxidation occurs through the degradation of phenolic hydroxyl groups, and the ammoxidation of etherified lignin structures occurs at a slower rate (Capanema *et al.*, 2001a; Fischer and Schiene, 2002), KRT and SOD were expected to be more reactive to ammoxidation and incorporate higher nitrogen contents compared to SLS.

Fourier Transform Infrared Spectroscopy

FTIR spectroscopy was used to provide more insight into the unique structural features of each raw material. The FTIR spectra of the raw lignins is shown in Figure 20, and the peak assignments are shown in Table 13. KRT and SOD lignin were characterized by a broad band at 3 400 cm^{-1} '(**1**, hydroxyl groups), and two peaks at 2 900 cm^{-1} (**2**, C-H stretching of methyl and methylene groups) and at 2 820 cm^{-1} (**3**, C-H vibrations of methoxyl groups). SLS did not exhibit any distinctive peaks at these wavenumbers, indicating that this raw material did not have appreciable quantities of phenolic hydroxyl, or methoxyl groups. This is in agreement with the finding of low phenolic hydroxyl group content (0.37 mmol/g) in SLS, obtained through ultraviolet spectroscopy (Table 12) and is in agreement with findings from previous studies (Govender, 2020; Du Toit , 2021).

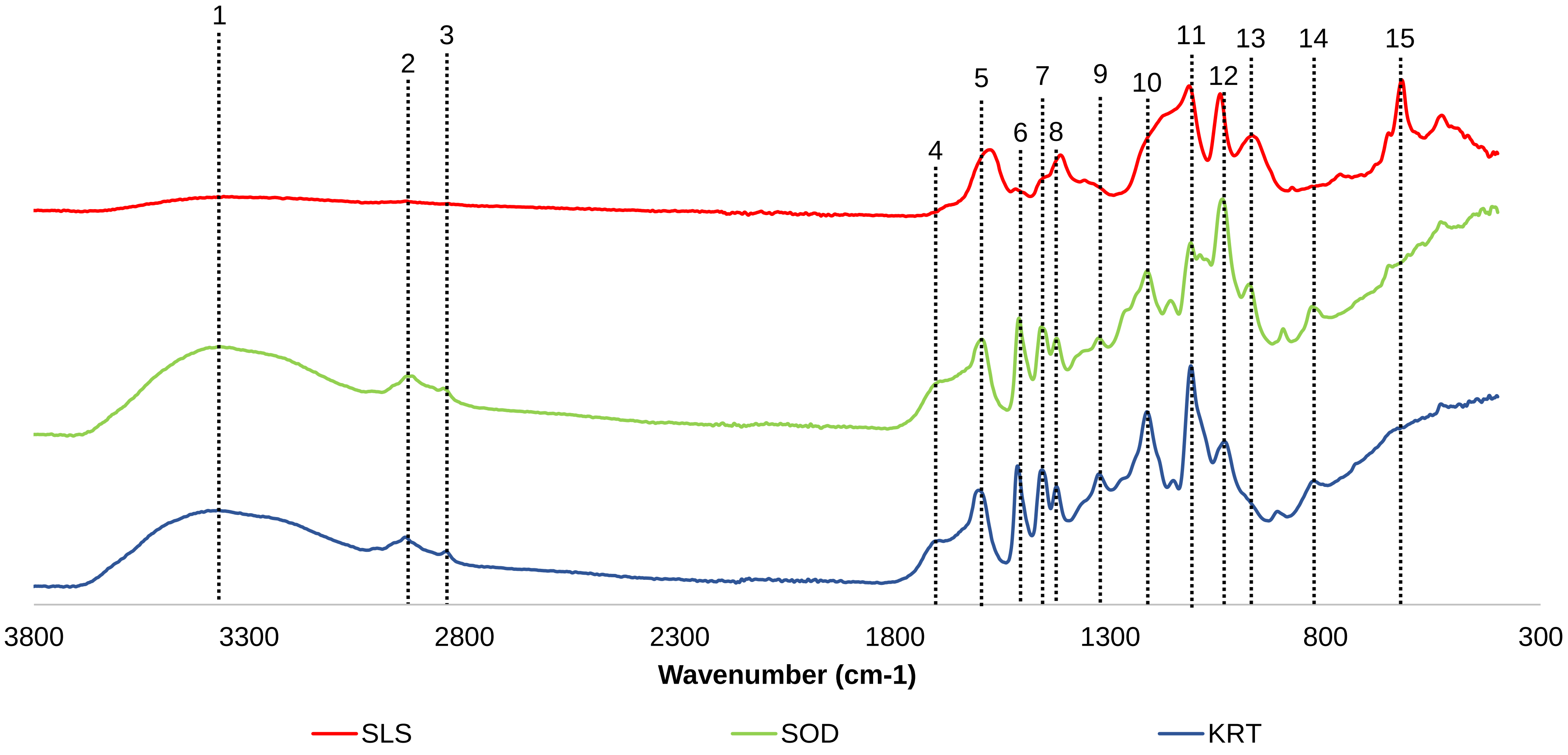


Figure 20: FTIR spectra of raw lignins

The incorporation of nitrogen into lignin is related to the oxidative degradation of phenolic hydroxyl groups and correlates linearly with the oxidative degradation of methoxyl groups (Capanema *et al*., 2001a; Fischer and Schiene, 2002). The abundance of these structural features in KRT and SOD provided more support to the expectation that these raw materials may be more reactive to ammoxidation in comparison to SLS.

The peak in the range 1 709 – 1 738 cm^{-1} (**4**, C=O stretch in unconjugated ketones and carbonyl groups) had the highest intensity in SOD, an appreciable intensity in KRT and a low intensity for SLS (Figure 20, Table 13). This peak is attributed to the functional groups present in carbohydrates and lignins. It had already been shown through UV spectroscopy that KRT and SOD had higher total sugar contents compared to SLS (Table 12), due to biomass origin and the severity of the pulping method. The appearance of peak **4** (1 709-1 738 cm^{-1}, C=O stretch in unconjugated ketones and carbonyl groups) thus confirmed earlier observations.

The peaks at 1 508 – 1 650 cm^{-1} (**5**, aromatic skeletal vibration) and at 1 505-1 515 cm^{-1} (**6**, aromatic skeletal vibration) (Figure 20, Table 13) are typical for lignin, and were observed in all three starting materials (Faix, 1991). The peak at 1 459 cm^{-1} (**7**, C-H deformations in methyl groups) is pronounced for SOD and KRT, but not for SLS, while the peak at 1 424 cm^{-1} (**8**, C-H deformations and aromatic skeletal deformation) is sharp for SOD and KRT, but broad for SLS. Peak **7** (1 459 cm^{-1}, C-H deformations in methyl groups) could have been shifted to lower wavenumbers in SLS due to the electrical density of the structure of SLS.

The peak at 1 325 – 1 330 cm^{-1} (**9**, C-O of S ring) is most pronounced in KRT than in SOD and is absent in SLS. KRT was obtained from hardwood biomass, which contains more S units compared to softwoods (Gellerstedt, 2009). SLS, which was obtained from a mixture of hardwood and softwood contained a smaller quantity of S units. Grasses contain S units in appreciable quantities, hence the abundance of S units in SOD (Beckham, 2018). Sodium lignosulphonate had a lower abundance of S units, which is the monolignol with the highest degree of methoxylation. This corresponded to absence of peak **3** in SLS which is attributed to C-H stretch in methoxyl groups.

The peak at 1 210 – 1 214 cm^{-1} (**10**, C-C ring stretching, C-H deformation, O-H deformation) had a sharp intensity in KRT and SOD but appeared as an absorption band in SLS. According to Faix (1991), for compounds containing hydroxyl groups, peak **1** is followed by an absorption band at 1 200 – 1 000 cm^{-1} which confirms the presence of phenolic hydroxyl groups. In this study, the presence of phenolic hydroxyl groups in KRT and SOD was also confirmed through ultraviolet spectroscopy (Table 12).

The peak at 1 112 – 1 118 cm^{-1} (**11**, C-H deformation or sulphate) was more pronounced in KRT and SLS, which are the sulphur containing lignins (Table 10, Figure 20, Table 13). This peak was less pronounced in SOD (Figure 20, Table 13) which had a lower sulphur content (0.3%) compared to KRT (6.3%) and SLS (6.2%), according to the elemental analysis (Table 10). The peak at 1 030 – 1044 cm^{-1} (**12**, aromatic C-H deformation and C-O stretching of primary alcohols) is also characteristic of lignin and was observed in all three raw materials. SLS may have contained an appreciable amount of aliphatic hydroxyl groups even though it had a negligible amount of phenolic hydroxyl groups. The peak observed at 966 – 990 cm^{-1} (**13**, Trans unsaturated vinyl groups) was most pronounced in SLS than in SOD and was absent in KRT. The peak at 835 cm^{-1} (**14**, C-H bending in position 2, 4, 6 of S units all positions in H units) was not present in SLS, but present in KRT and SOD, confirming that SLS did not have an appreciable quantity of S units, and hence a low quantity of methoxyl groups. The peak at 622 cm^{-1} (**15**, sulphate ion) was only present in SLS, which was expected to contain an appreciable quantity of sulphate ions, originating from the pulping method.

Table 13 : Assignment of the IR peaks for the spectra shown in figure

Peak	Wavenumber (cm^{-1})	Assignment	Reference
1	3 412 – 3 460	O-H stretch	(Faix, 1991)
2	3 000 – 2 842	C-H stretch in methyl and methylene groups	(Faix, 1991)
3	2 820 – 2 810	C-H stretch of Methoxyl groups (CH_3-O-)	(Nandiyanto *et al.*, 2019)
4	1 738 – 1 709	C=O stretch in unconjugated ketones and carbonyl groups	(Faix, 1991)
5	1 593 – 1 605	Aromatic skeletal vibrations and C=O stretch	(Faix, 1991)
6	1 505 – 1 515	Aromatic skeletal vibrations, G>S	(Faix, 1991)
7	1 460 – 1 470	C-H deformations; asymmetric in methyl groups and –CH2-	(Faix, 1991)
8	1 422 – 1 430	Aromatic skeletal vibrations combined with C-H in plane deformation	(Faix, 1991)
9	1 325 – 1 330	C-O stretching of S ring	(Faix, 1991)
10	1 210 - 1 214	C-C ring stretching, C-N stretching, C-H deformation, O-H deformation	(Bykov, 2008; Nandiyanto *et al.,* 2019)
11	1 112-1 118	C-H deformation or sulphate	(Namane, 2016)
12	1 030 - 1 044	Aromatic C-H in plane deformation, C-O deformation in primary alcohols	(Bykov, 2008; Nandiyanto *et al.,* 2019)
13	966 – 990	-HC=CH- out of plane deformation	(Faix, 1991)
14	834 – 835	C-H out of plane in position 2 and 6 of S, and in all positions of H units	(Faix, 1991)
15	680 – 610	Sulphate ion	(Nandiyanto *et al.*, 2019)

4.2 N-lignin characterization

N-lignins produced from technical lignins via either ammoxidation only, or a combination of pre-oxidation with subsequent ammoxidation, were characterized to determine their suitability for use as soil amendments. The prefix “N-” denotes samples that were ammoxidized without pre-oxidation, “N-$O_{1.5}$-” denotes samples that were ammoxidized after pre-oxidation with 1.5% H_2O_2, and “N-O_5-” denotes samples that were ammoxidized after a pre-oxidation with 5% H_2O_2. In the present study, low pressure air (10 barg) was used as the oxidant for ammoxidation in a Parr reactor, as opposed to pure oxygen (Meier *et al*., 1994; Capanema *et al*., 2001a; Ramírez *et al.*, 2007) or air at atmospheric pressure (Tyhoda, 2008) used in previous studies.

Total Nitrogen content and C/N

The C/N ratio is the most important parameter in characterizing N-lignins for agricultural applications because it gives an indication whether the application of the N-lignin is likely to cause a net-mineralization or a net-immobilization of nitrogen in the soil (Fischer and Schiene, 2002). It has been reported that N-lignins with a C/N less than 20 are suitable for use as soil organic amendments (Meier *et al*., 1994). N-lignins are characterized by an increased nitrogen content, increased oxygen content, and a decreased carbon content compared to raw lignins (Ragab, 2008; Tyhoda, 2008). The elemental composition of ammoxidized lignins produced in this study is shown in Table 14 .

Table 14 : Elemental composition of ammoxidized lignins

	C [%]	N [%]	H [%]	S [%]	C/N
KRT	56.30	0.20	6.50	6.30	281.50
N-KRT	51.20	2.80	6.70	5.10	18.29
N-$O_{1.5}$-KRT	46.20	3.20	7.10	5.70	14.44
N-O_5-KRT	49.48	4.02	6.15	5.64	12.31
SOD	56.40	0.30	7.40	0.30	188.00
N-SOD	53.20	1.90	7.40	0.40	28.00
N-$O_{1.5}$-SOD	51.70	2.10	7.60	1.80	24.62
N-O_5-SOD	52.82	3.34	6.64	0.92	15.81
SLS	28.30	0.20	3.60	6.20	141.50
N-SLS	28.00	0.40	3.50	8.78	70.00
N-$O_{1.5}$-SLS	24.00	0.50	3.20	5.70	48.00
N-O_5-SLS	23.57	1.61	2.73	6.31	14.64

Ammoxidation increased the nitrogen contents in all three raw materials, corresponding to a decrease in their C/N ratios, as expected (Table 14). The ammoxidation of KRT increased the nitrogen content from 0.2% (KRT) to 2.8% (N-KRT). This decreased the C/N ratio from 281.5 to 18.3, meeting the criterion for use as a soil amendment (C/N<20). On the contrary, despite an improvement in the total nitrogen content from 0.30% to 1.90%, N-SOD had a C/N ratio of 28, which does not meet the criterion. The nitrogen content of SLS increased to 0.4% after ammoxidation, and the material had a C/N ratio of 70, which was still too high (Table 14). It was thus clear that KRT and SOD were more reactive to ammoxidation compared to SLS, an outcome that was predicted from the high phenolic hydroxyl group content and high methoxyl group content of these raw materials compared to SLS (Table 12, Table 13, Figure 20). Kraft lignin had the highest phenolic group content, and was the most reactive material to ammoxidation (Table 12, Table 14).

However, the amount of nitrogen incorporated into all three raw materials was lower than anticipated. For example, Tyhoda (2008) reported that the ambient pressure ammoxidation of Sucrolin^{TM} (auto hydrolysis bagasse lignin), Indulin^{TM} (purified unsulphonated kraft lignin) and calcium lignosulphonate from LignoTech yielded N-lignins containing 6.25% N, 3.15% N, and 3.36% N, respectively. Higher nitrogen contents were expected in this study, due to the use of pressurized air (Fischer and Schiene, 2002). The lower reactivity of the lignins used in the present study may be attributed to the differences in functional group composition of industrial lignins compared to commercial lignins. Balakshin and Capanema (2015) showed that Indulin^{TM} had lower molecular weights and higher phenolic group contents compared to hardwood kraft lignin, while Sucrolin^{TM} contained more carboxyl groups and more oxygenated aliphatic structures compared to soda bagasse lignins. Highly oxidized technical lignins are more reactive to ammoxidation (Tyhoda, 2008). Furthermore, the use of a Parr reactor with air as the oxidant may have not reached the extent of ammoxidation similar to that reached in an immersion jet setup or high-pressure oxygen used in previous studies (Meier *et al.,*1994; Ramírez *et al.*, 2007; Tyhoda, 2008; Tyhoda *et al.*,2012).

The reactivities of the selected lignins to ammoxidation could be ranked in the following order; KRT > SOD > SLS, based on their nitrogen incorporation (Table 14). Meier *et al.*, (1994) reported that the reactivity of commercial lignins to ammoxidation decreased as follows; sodium lignosulphonate > kraft lignin > soda lignin. The difference in the reactivity of the sodium lignosulphonate may be attributed to the difference in the pulping methods used in the isolation of this material. Under acid sulphite pulping, lignin undergoes a severe fragmentation (compared to NSSC), which increases the number of phenolic hydroxyl groups through which nitrogen is incorporated (Capanema *et al.*, 2001; Matsushita, 2015; Li *et al.*, 2018). Moreover, SLS was found to have a much lower carbon content, and a considerably higher ash content (Table 10) compared to the sodium lignosulphonate used in literature (Meier *et al.*, 1994). This indicates that this material had a lower quantity of organic substrate onto which nitrogen could be bound, resulting in a significantly lower reactivity to ammoxidation.

Since the N-lignins obtained in this study had lower nitrogen contents and higher C/N ratios compared to N-lignins reported previously (Table 14), it was necessary to pretreat the starting materials with either 1.5% or 5% hydrogen peroxide to improve the nitrogen enrichment, especially for SLS and SOD. Pre-oxidation degrades the substrates and increases the number of oxygen rich functional groups through which nitrogen is bound (Capanema *et al.*, 2001).

Oxidation of the starting materials with 1.5% hydrogen peroxide prior to ammoxidation increased the amount of nitrogen incorporated into KRT from 2.8% to 3.2% (N-$O_{1.5}$-KRT) yielding a C/N ratio of 14.44, while the amount of nitrogen incorporated onto SOD increased from 1.9% to 2.1% (N-$O_{1.5}$-SOD) (Table 14). Similar improvements in nitrogen incorporation have been reported in the literature. Tyhoda (2008) reported that pre-treating a bagasse lignocellulosic residue with hydrogen peroxide improved the amount of nitrogen incorporated during ammoxidation from 2.64% to 3.08%. In this study, however, this pretreatment was not sufficient to produce N-lignins with desirable C/N ratios from SOD and SLS. N-$O_{1.5}$-SOD had a C/N ratio of 24.62, and N-$O_{1.5}$-SLS still had a high C/N ratio of 48 (Table 14). Stronger oxidative conditions were thus necessary in the pre-oxidation to improve nitrogen incorporation.

N-lignins with a C/N less than 20 were obtained from all three starting materials after employing a higher oxidant concentration (5% H_2O_2) in the pretreatment. The N-lignins obtained from KRT, SOD, and SLS had nitrogen contents of 4.02%, 3.34% and 1.61%, respectively (Table 14). This corresponded to C/N ratios of 12.31, 15.81 and 14.64, respectively. Increasing the oxidant concentration may have led to severe oxidative modification in the substrates, increasing the number of active sites for nitrogen incorporation (Tyhoda, 2008). The application of these products can be expected to cause a net-mineralization of nitrogen in the soil (Meier *et al.*, 1994). According to Li *et al.* (2021), organic fertilizers and soil amendments used in Massachusetts have an average C/N ratio of 16.9. Thus, these N-lignins may even be superior to some commercial soil organic amendments.

Phenolic hydroxyl group content

The oxidative degradation of phenolic groups is desirable for ammoxidation. Pre-oxidation with hydrogen peroxide, both at 1.5% and 5%, resulted in the degradation of more phenolic hydroxyl groups in KRT and SOD, compared SLS, leading to an increase in nitrogen enrichment (Figure 21, Table 14). Northey (2001) reported that lignin structures that contain more phenolic hydroxyl groups and methoxyl groups have a higher reactivity to oxidation. Thus, the lower reactivity of SLS could be predicted from a lower abundance of these functional groups (Table 12, Table 13, and Figure 20). Pre-oxidation with 1.5% hydrogen peroxide resulted in the degradation of 1.64 mmol/g, 0.69 mmol/g, and 0.26 mmol/g phenolic hydroxyl groups in KRT, SOD, and SLS, respectively (Figure 21). Increasing the hydrogen peroxide concentration to 5% increased the phenolic hydroxyl group degradation in the starting materials to 3.27 mmol/g, 1.04 mmol/g, and 0.33 mmol/g, respectively (Figure 21). Capanema *et al.* (2001) reported that about 60% of the total nitrogen incorporated onto lignin is associated with the degradation of phenolic hydroxyl groups. The incorporation of nitrogen into phenolic lignin moieties also occurs at a faster reaction rate compared to non-phenolic moieties, due to their ease of oxidation (Capanema *et al.*, 2001a; Capanema *et al.*, 2001b; Northey, 2001). Nitrogen enrichment was improved through the oxidation of more phenolic hydroxyl groups, increasing the nitrogen binding sites in the starting materials (section 2.5.3).

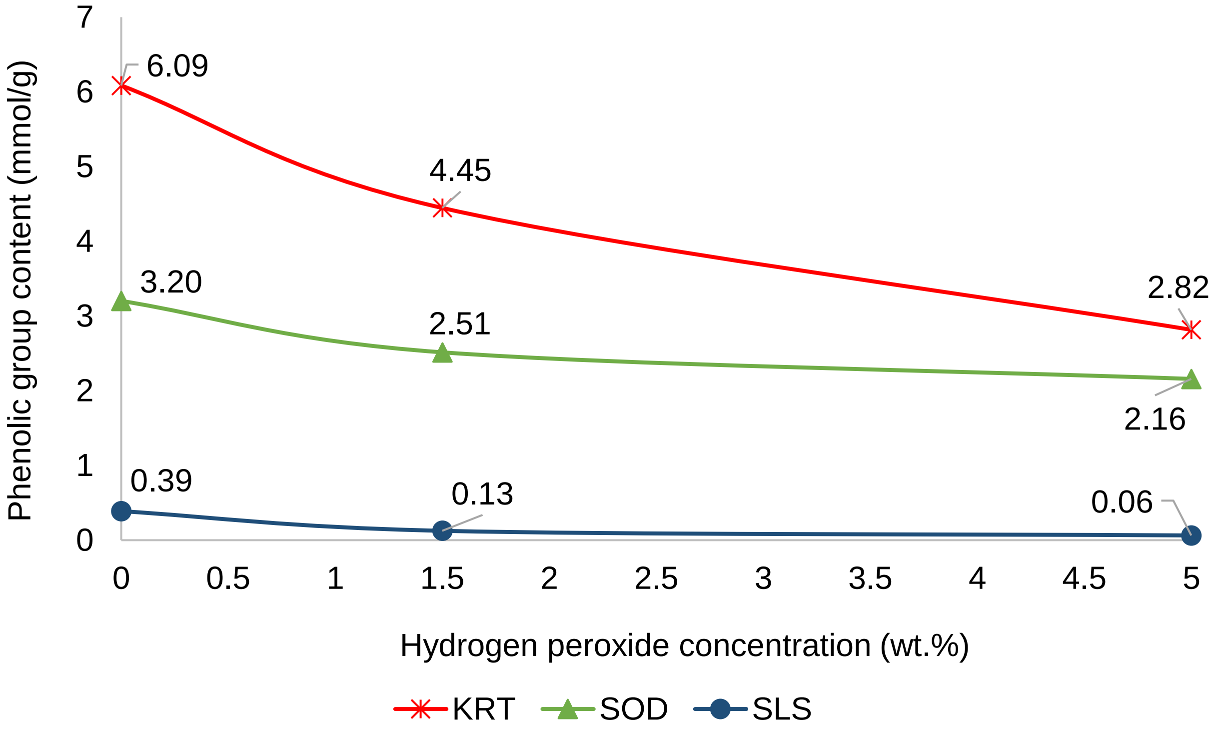


Figure 21: Phenolic hydroxyl group content of pre-oxidized lignins

Fourier Transform Infrared Spectroscopy

Structural analysis of N-lignins using FTIR confirmed the degradation of phenolic groups and methoxyl groups in KRT and SOD after ammoxidation, accompanied by the incorporation of nitrogen-rich functional groups, while SLS did not undergo appreciable structural changes. The FTIR spectra are shown in Figure 22 to Figure 24, and the peak assignments are shown in Table 15.

The peak at 3400 cm^{-1} (**1**, phenolic hydroxyl groups) was degraded and shifted to lower wavenumbers as the extent of pre-oxidation was increased in KRT (Figure 22) and SOD (Figure 23). This was a result of the degradation of hydroxyl groups and overlapping with the N-H stretch in amines and ammonium salts (Capanema*, et al*., 2001a). There was an emergence of a peak at 3 190 cm^{-1} (**2**, N-H stretch in secondary amines) as the extent of pre-oxidation increased. This provided evidence for the incorporation of amine groups into these lignins (KRT and SOD). The peaks assigned to methylene groups (**3**, C-H stretch in methylene groups) and to methoxyl groups (**4**, C-H stretch in methoxyl groups) decreased in intensity as the extent of pre-oxidation increased in KRT and SOD. This confirmed that these functional groups undergo oxidative degradation during ammoxidation (Fischer and Schiene, 2002). No evidence for the incorporation of nitrile groups was observed at 2 210 – 2 260 cm^{-1} (peak **5**). This was in agreement with findings by previous scholars (Meier *et al.*, 1994; Ramírez *et al.*, 2007).

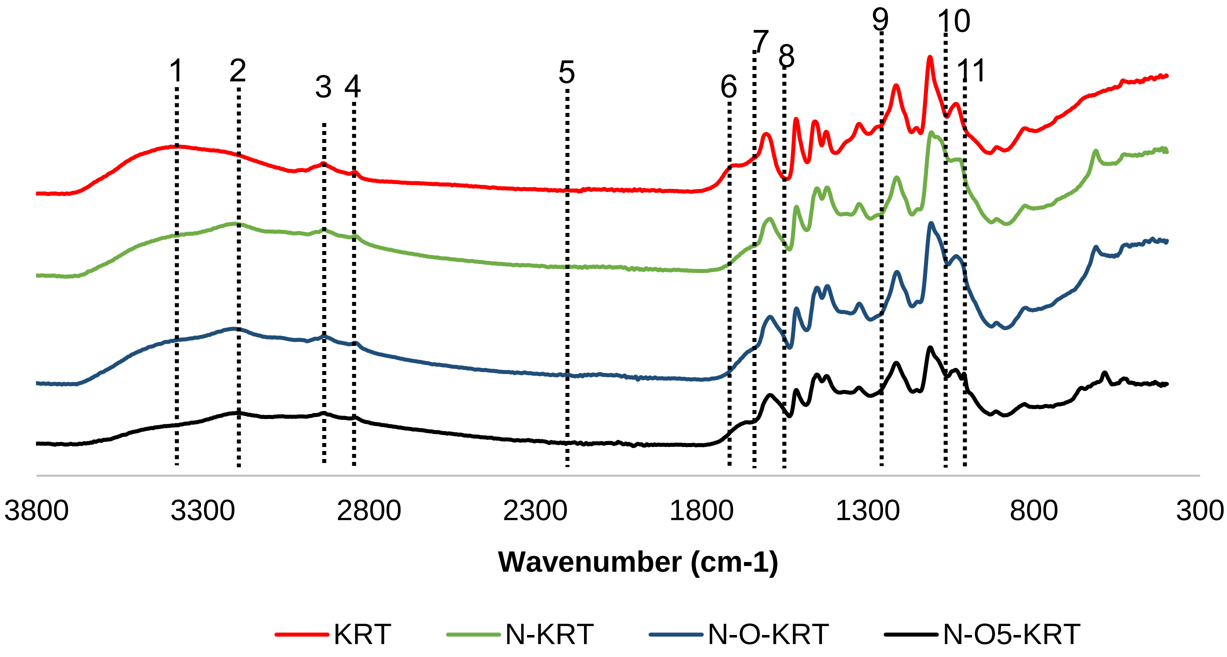


Figure 22: FTIR Spectra of raw and ammoxidized kraft lignin

Peak **6** (C=O stretch in unconjugated ketones, carbonyls and in ester groups) was altered slightly in KRT and SOD, indicating the modification of carbohydrates during ammoxidation, which may form phytotoxic substances (Klinger *et al.*, 2013). The effect of this modification was assessed in a nutrient uptake and plant toxicity trial (chapter 5). The intensity of peaks at 1 593 – 1 605 cm^{-1} (**7**, Aromatic skeletal vibrations and C=O stretch) and at 1 505 – 1 515 cm^{-1} (**8**, aromatic skeletal vibrations) decreased in KRT and SOD. This corresponded to an oxidative degradation of aromatic rings as ammoxidation proceeded (Qi-Pei *et al.*, 2006; Ramírez *et al.*, 2007). The peak at 1 030 – 1 044 cm^{-1} (**10**, Aromatic C-H in plane deformation, C-O deformation in primary alcohols) decreased in all three lignins due to the oxidative degradation of primary alcohols.

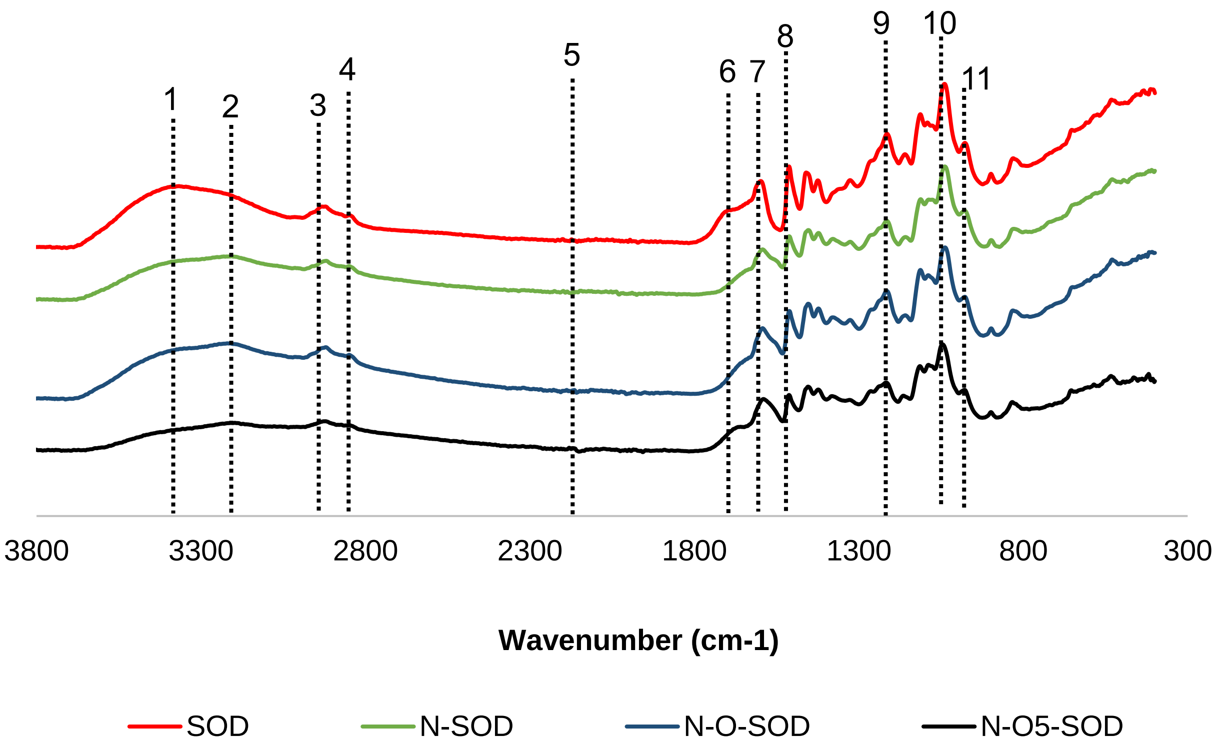


Figure 23: FTIR Spectra of raw and ammoxidized soda lignin

Interestingly, SLS did not undergo appreciable structural modifications even with an increase in pre-oxidation. This was contrary to the expectation that lignosulphonates have a high reactivity to ammoxidation as reported in literature (Meier *et al*., 1994). This was due to a lower quantity of phenolic groups in the SLS used in this study, as a result of the biomass origin, pulping method, and low organic matter content (Table 10, Table 13,Table 15, Figure 20, and Figure 24). The major changes in this raw material were the narrowing of the band at 1112 – 1118 cm^{-1} (C-H deformation or sulphate), which may be due to the disappearance of the band at 1210-1214 cm^{-1} (**9**,C-C ring stretching, C-H deformation, O-H deformation), due to oxidative degradation of aliphatic hydroxyl groups, and the disappearance of the peak at 966 – 990 cm^{-1} (**11**, -HC=CH- out of plane deformation) due to the oxidation of vinyl structures. No evidence for the incorporation of nitrogen functional groups could be obtained from the FTIR spectra, due to low nitrogen contents (Table 14).

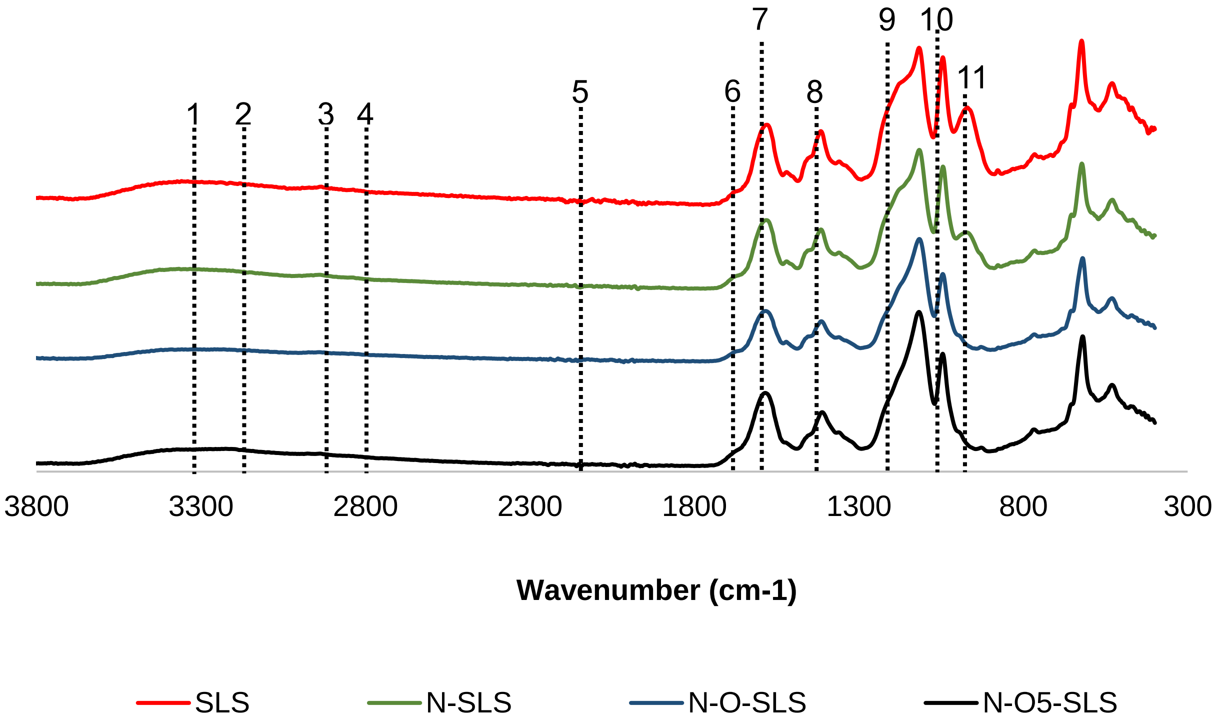


Figure 24: FTIR Spectra of raw and ammoxidized sodium lignosulphonate

Table 15: Assignment of the IR peaks for the spectra shown in Figures 22 - 24

Peak number	Wavenumber (cm^{-1})	Assignment	Change during ammoxidation		
			KRT	SOD	SLS
1	3412 – 3460	O-H stretch	⟵	⟵	⟷
2	3180 - 3190	N-H stretch in secondary amines	⟶	⟶	⟷
3	3000 – 2842	C-H stretch in methyl and methylene groups	⟵	⟵	⟷
4	2820 – 2810	C-H stretch of methoxyl groups (CH_3-O-)	⟵	⟵	⟷
5	2210 - 2260	C≡N stretch in nitriles	⟵	⟵	⟷
6	1738 – 1709	C=O stretch in unconjugated ketones, carbonyls and in ester groups (from carbohydrates)	⟵	⟵	⟷
7	1593 – 1605	Aromatic skeletal vibrations and C=O stretch	⟵	⟵	⟷
8	1505 – 1515	Aromatic skeletal vibrations, G>S	⟵	⟵	⟷
9	1210 - 1214	C-C ring stretching, C-H deformation, O-H deformation	⟷	⟷	⟵
10	1030 - 1044	Aromatic C-H in plane deformation, C-O deformation in primary alcohols	⟵	⟵	⟵
11	966 - 990	-HC=CH- out of plane deformation	⟷	⟷	⟵

⟵: Decrease, ⟶: Increase, ⟷: No change

Distribution of nitrogen functional groups

The N-lignins obtained in this study were characterized by higher proportions of inorganically bound nitrogen (53% to 71%) and lower nitrogen contents compared to N-lignins reported in literature (organic nitrogen contents ranged from 10% to 50%) (Fischer and Schiene, 2002) (Figure 25, Table 14). This was not a desirable outcome since the goal of ammoxidation is to minimize the proportion of inorganically bound nitrogen and maximize the proportion of organically bound nitrogen, which is made plant-available over an extended period in the soil (Fischer and Schiene, 2002; Tyhoda, 2008). This was a result of a lower extent of oxidation imparted on the lignins.

Strongly oxidative conditions increase the number of active sites where nitrogen can be incorporated, and increase the extent of recombination radical reactions which increase the proportion of organically bound nitrogen (Fischer and Schiene, 2002; Tyhoda, 2008). The proportion of organically bound nitrogen in N-lignins after ammoxidation increased with increasing hydrogen peroxide concentration in the pre-oxidation, in all three starting materials (Figure 25). For example, the content of inorganically bound nitrogen for KRT increased from 2% in N-KRT to 2.53% in N-O_5-KRT, while its proportion decreased from 71% in N-KRT to 65% in N-O_5-KRT (Figure 25,Table 14). This complements findings reported in literature. Tyhoda (2008) reported that the pre-oxidation of Sucrolin^{TM} using 5% H_2O_2 followed by ammoxidation using high pressure oxygen increased nitrogen incorporation and decreased ammonium bound nitrogen (inorganic) from 38.42% to 34.76%.

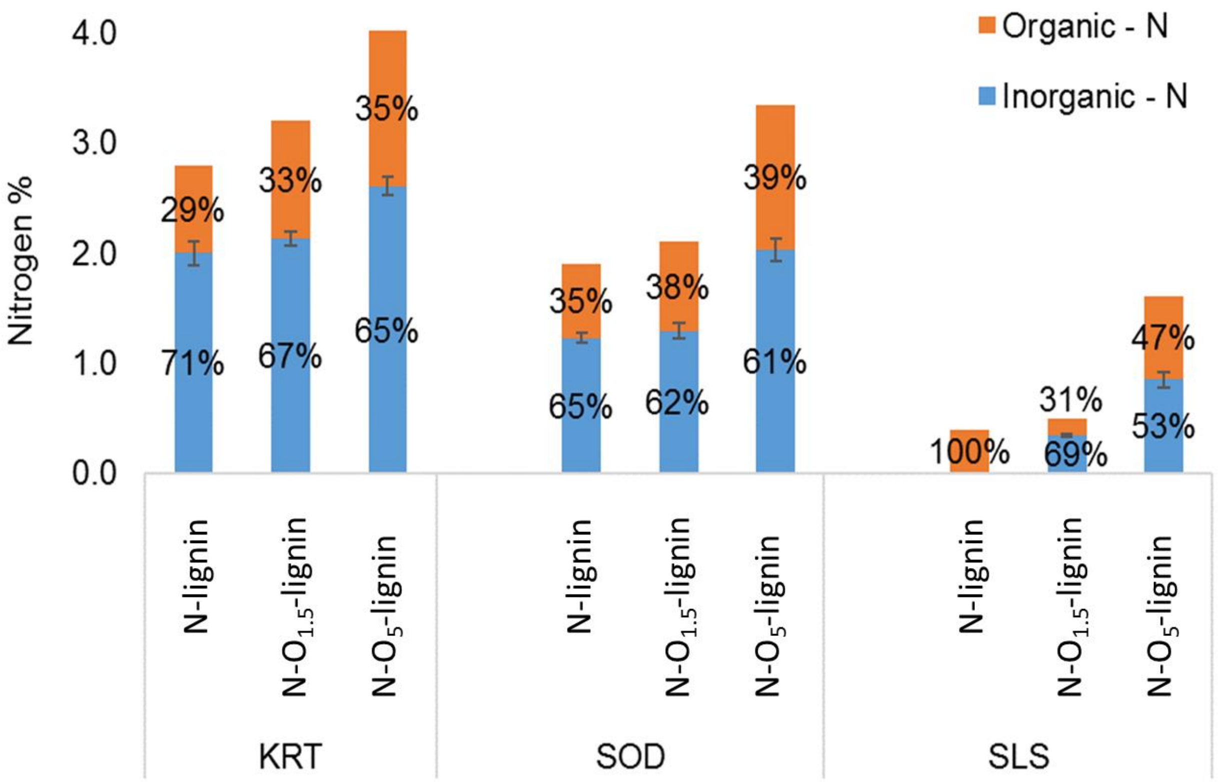


Figure 25: Distribution of nitrogen functional groups

Acidity, Electrical conductivity, salt index and solubility

N-O_5-lignins obtained from KRT (pH 5.48) and SOD (pH 6.51) were slightly acidic, while the N-O_5-lignin obtained from SLS (pH 8.81) was alkaline (Table 16). Soil amendments can affect the soil pH and salinity, which affects plant nutrient uptake and the livelihood of soil microorganisms (Li *et al*., 2021). A soil pH between pH 5 and pH 7 is beneficial for both soil microorganisms and plants (Ngezimana and Agenbag, 2014; Tataru-Farmus *et al*., 2018). The application of N-O_5-KRT (pH 5.48) and N-O_5-SOD (pH 6.51) on neutral soil may not change the pH beyond the optimum range (pH 5 to pH 7), as opposed to N-O_5-SLS (pH 8.81) (Table 16). However, N-O_5-SLS may be used as an amendment to improve the pH of strongly acidic soil. Sodium lignosulphonate had a high content of sodium (144 900 mg/kg) (Table 11) derived from residual pulping chemicals, which forms an alkaline solution when dissolved in water. Kraft lignin and SOD contained a higher abundance of carboxylic acid and phenolic groups in their structures, and lower quantities of sodium (Table 12, Figure 22, Figure 23, Figure 24, and Table 15). These materials were thus not expected to be alkaline (Ragnar *et al.*, 2000; Zhang and Flaherty, 2020).

Table 16 : Acidity, electrical conductivity, salt index and solubility of N-O_5-lignins

	pH	Electrical conductivity (dS/m)	Salt index (%)	Solubility (%)
N-O_5-KRT	5.48	0.591	16.53	42.0
N-O_5-SOD	6.51	0.369	10.32	43.6
N-O_5-SLS	8.81	2.27	63.62	99.7

N-O_5-KRT (0.591 dS/m, 16.53% SI) and N-O_5-SOD (0.369 dS/m, 10.32% SI) had considerably lower electrical conductivities (EC) and salt indexes (SI) compared to N-O_5-SLS (2.27 dS/m, 63.62% SI) (Table 16). N-O_5-KRT and N-O_5-SOD were anticipated to have lower SI compared to N-O_5-SLS due to a lower ash content in the raw materials (Table 10, Table 11). The salt index of N-O_5-SLS was considerably high even compared to commercial fertilizers such as di-ammonium phosphate (29.2%) and potassium sulphate (42.6%) (Laboski, 2008). A high salt concentration in the soil solution can affect plant growth through increasing the soil osmotic pressure, depriving plants of water, and through causing ion competition with essential plant nutrients (Na^+/Ca^{2+}, Na^+/K^+, and Cl^-/NO_3^-) (Kopittke *et al.*, 2019). Most vegetables have a salinity threshold of 1 to 2.5 dS/m in saturated soil extracts (Machado and Serralheiro, 2017). N-O_5-SLS had an EC that is on the upper end of the range for most vegetables and may cause growth inhibition to sensitive plants (Table 16) (Machado and Serralheiro, 2017; Kopittke *et al.*, 2019).

N-lignins obtained from KRT (42%) and SOD (43.6%) were partially soluble in water (Table 16). The presence of sulphonic groups in the chemical structure of lignosulphonate makes them highly water soluble (Naron *et al.*, 2017), thus, the high solubility of N-O_5-SLS was anticipated. On the other hand, the solubility of N-O_5-KRT and N-O_5-SOD could be ascribed to the impartation of hydrophilic functional groups onto the lignin structure and the degradation of hydrophobic structures during ammoxidation (Figure 22, Figure 23, Figure 24, and Table 15) (Capanema *et al.*, 2001b; Ragab, 2008).

However, Capanema *et al.* (2001a) reported that organosolve lignin was completely solubilized after 165 minutes when ammoxidized at 130 °C using 12 bar oxygen. Lower extents of nitrogen incoporation and solubilization were achieved in this study using air instead of pure oxygen as the oxidant.

Water retention capacity

The application of N-O_5-KRT, N-O_5-SOD, and N-O_5-SLS significantly ($p < 0.05$) improved the water retention capacity of sandy soil. While the control retained only 31.4% of the initial water mass, sand treated with N-O_5-KRT, N-O_5-SOD, and N-O_5-SLS retained 51.4%, 46.5% and 63.5% of the initial water mass, respectively (Figure 26). The water retention capacity of these materials was also ascribed to the presence of hydrophilic functional groups in their chemical structures (Figure 22, Figure 23, Figure 24, and Table 15). N-O_5-SLS was expected to have a high water retention capacity due to its hydrophilicity, this is one of the properties that allows it to be used as a dust suppressant (Xu *et al*., 2018; Guo, 2018; Zhang *et al.*, 2020). Materials with a high water retention capacity may also improve the soil's resistance to wind erosion (Ding *et al.*, 2018), and improve the water use efficiency of crops (Li *et al.*, 2021), which is a desirable property for soil conditioning materials.

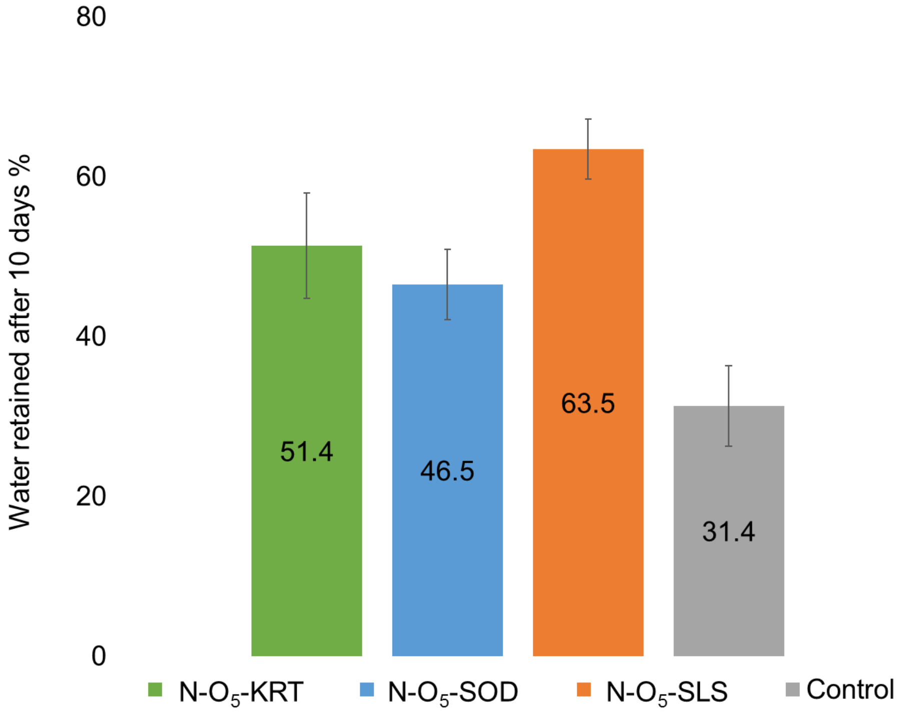


Figure 26: Percentage of water retained in N-O_5-lignins after 10 days

5 EFFECT OF N-O_5-LIGNIN ON THE GROWTH AND NITROGEN UPTAKE OF WHITE MUSTARD SEEDLINGS (*SINAPIS ALBA*)

5.1 Introduction

Characterization of the synthesized N-O_5-lignins revealed that they all had C/N less than 20 (Table 14), as such, these products were deemed suitable for use as soil amendments (Meier *et al.*, 1994). However, N-O_5-SLS (pH 8.81) had a pH that is beyond the optimum range for plant growth (pH 5 to pH 7), and a considerably high salt index (63.62%) compared to N-O_5-KRT (16.53%) and N-O_5-SOD (10.32%) (Table 16), making it susceptible to causing plant growth inhibition (Ngezimana and Agenbag, 2014; Machado and Serralheiro, 2017). In this section, all three N-O_5-lignins were tested in a plant trial to provide insight into the suitability of these products for soil amendment from a plant trial perspective. White mustard was selected for this assessment and was cultivated over a 4-week period. Braco white mustard seeds (*Sinapis alba*) were obtained from Agricol, Cape Town for use in this study. This crop was selected because of its fast growth rate and the simplicity in height measurement. The nursery at the Wood Science department, at Stellenbosch University was used for the trial. The temperature variation at the nursery during the cultivation period is shown in the appendix (Figure 35).

5.2 Materials and methods

5.2.1 Sand characterization

Malmesbury river sand was used for the cultivation of the seedlings. The sand was characterized at the laboratories of the Department of Agriculture in the Western Cape at Elsenburg according to standard methods reported by the Non-affiliated Soil Analysis Work Committee (1990). The methods used are identified in Table 17.

Table 17 : Soil characterization methods

Property	Method
pH	Potassium chloride extract
Total acidity	Potassium sulphate extract
Exchangeable K, Ca, Mg, Na	Ammonium acetate extract, read on ICP OES
Extractable B, Cu, Zn, and Mn	Ethylenediaminetetraacetic acid (EDTA) extract, read on ICP OES
Organic C	Walkeley-Black method
Extractable P	Citric acid extract, read on ICP OES
N	Kjeldahl method, read on a gallery discrete analyzer

Field capacity

The field capacity is the mass of water that the soil is able to hold without leaching. This parameter was determined by adding 100 ml of water to 100 g of oven dry sand and allowing it to drip into a volumetric flask until no further leaching could be observed. The top of the funnel was closed with a watch glass to avoid evaporation, and the setup was allowed to stand for an hour. The amount of water contained within the soil was determined by difference and used to calculate the field capacity. The measurement was conducted in triplicate.

5.2.2 Cultivation

Fifteen plant pots (height = 12 cm, cross-sectional area = 176.71 cm^2) were filled with 1500 g ± 1 g of Malmesbury river sand. Twenty-one seeds were planted in each pot, using a spacing of 2.5 cm (Tyhoda, 2008). Each pot was irrigated with 50 ml tap water every two to three days. The seeds were allowed to germinate and grow for a period of 10 days. After 10 days, 18 seedlings which had a height of ~ 45 mm were selected in each pot and allowed to grow further. The N-lignins were applied to the pots with irrigation water at a rate of 5 g/pot for three days over a period of five days, amounting to a total of 15 g N-lignin per pot (1% of the soil mass) for each treatment. The pots used as the control were irrigated with tap water only. Each treatment was completed in triplicate. The pots were placed randomly in the nursery to avoid statistical bias. Phosphorus and potassium were supplied through the application of mono-potassium phosphate ($KHPO_4$) at an application rate of 60 kg/ha and 76 kg/ha, respectively (Raghuvanshi *et al*.,2018). Wonder ^{TM} was used as the commercial reference fertilizer. The chemical composition of this product is shown in the appendix (Table 20). The nutrient application data for the trial is shown in Table 18.

Table 18 : Soil fertilizing material application rates

Treatment	Dosage	N_{tot} (wt.% sample)	N – applied (mg/pot)	NH_4-N (mg/pot)	KH_2PO_4 (mg/pot)
N-O_5-KRT	15 g/pot	4.02	603	391.95	465
N-O_5-SOD		3.34	501	306.11	465
N-O_5-SLS		1.61	241.5	128	465
Commercial reference	8 ml/pot	81.45 g – N/ℓ	651.6	651.6	-
Control	-	-	-	-	-

The mono-potassium phosphate dosage, total nitrogen applied per pot, and ammonium nitrogen available per pot were calculated as shown in Equations 9 - 11;

- **Mono-potassium phosphate**

$$\mathrm{KHPO_4(mg/pot)} = \frac{\mathrm{NA\ x\ CA}}{\mathrm{MF}}$$ **Equation 9**

Where;

NA: Nutrient application rate (mg per ha)

CA: Cross sectional area (ha)

MF: Mass fraction of nutrient in mono-potassium phosphate

- **Total nitrogen applied**

$$\text{Ntot (mg/pot)} = \text{N\% Material} \times 15\ \text{g} \times 1\,000 \qquad \textbf{Equation 10}$$

- **Ammonium nitrogen available**

$$\text{NH}_4\text{-N (mg/pot)} = \text{NH4-N \% Material} \times 15\ \text{g} \times 1\,000 \qquad \textbf{Equation 11}$$

5.2.3 Growth rates

The heights and diameters of the seedlings were measured weekly, using vernier calipers. Measurements were taken from all three pots for each treatment and the results were reported to show averages and standard deviations.

5.2.4 Fresh mass yield and protein yield

The fresh mass and protein mass yield were determined at the end of the four-week cultivation period (from the date on which the N-lignins were applied). The seedlings were cut 2 mm above the soil surface, weighed to determine the fresh mass yield, and oven-dried at 40 ^{o}C until constant mass to determine the dry mass yield. This drying temperature was selected to avoid excessive nitrogen loss (Tyhoda, 2008). The fresh mass yield was calculated using Equation 12 below;

$$\text{Fresh mass yield (t/ha)} = \frac{\text{Fresh mass harvested (g/pot)}}{\text{Surface area of pot (ha)}} \times \frac{\text{1 ton}}{\text{1 000 000 g}} \qquad \textbf{Equation 12}$$

The dried plant material was analyzed for elemental composition as described in section 3.3. The protein mass yield was calculated from the dry mass as shown in Equation 13;

$$\text{Protein mass yield (kg/ha)} = \frac{\text{Dry mass harvested (g)}}{\text{Surface area of pot (ha)}} \times 6.25 \times \text{Nitrogen \%} \qquad \textbf{Equation 13}$$

5.2.5 Nutrient Use efficiency

The nutrient use efficiency (NUE) was calculated according to the difference method reported by Baligar *et al.*, (2001) using equation 14;

$$\text{NUE} = \frac{\text{DMF} \times \text{NF} - \text{DMN} \times \text{NN}}{\text{AF}} \times 100 \qquad \textbf{Equation 14}$$

Where;

DMF: Dry mass yield of fertilized crops (g)

NF: Nitrogen content of fertilized crops (%)

DMN: Dry mass yield of crops with no fertilizer (g)

NN: Nitrogen content of crops with no fertilizer (%)

AF: Mass of fertilizer applied (g)

5.3 Results and Discussion: Plant trial assessment

5.3.1 Sand characterization

The properties of Malmesbury sand are shown in Table 19, and were compared to the critical levels for nutrient deficiency as reported by Ngezimana and Agenbag, (2014). The pH of the soil (5.9) was within the optimal range for plant growth: pH 5 to pH 7 (Ngezimana and Agenbag, 2014) (Table 19). The application of N-O_5-KRT (pH 5.48) and N-O_5-SOD (pH 6.51) may not cause considerable changes to the soil pH compared to N-O_5-SLS (pH 8.81), which had a considerable difference to the pH of this soil (2.91 pH units) (Table 16). The macro- and micronutrient contents of the soil were below the critical levels for nutrient deficiency for all nutrients except for copper (actual level: 0.71 mg/kg, critical level: < 0.3 mg/kg) and zinc (actual level: 0.65 mg/kg, critical level: < 0.5 mg/kg), while the sodium content was below toxic levels (actual level: 7 mg/kg, critical level: > 250 mg/kg) (Table 19). However, the application of N-O_5-SLS which had a high sodium content (144 900 mg/kg) would be expected to increase the sodium content of the sand beyond the critical level (Table 11). The low nutrient and organic carbon (0.07%) content status of the sand was a desirable property, because the nutrient status of the sand would not interfere with the results of the trial (Table 19). The field capacity (20 ml/100 g dry sand) was sufficiently high to avoid leaching for an irrigation rate of 50 ml/1500 g - sand every two to three days (Table 19).

Table 19 : Field capacity, pH, and nutritional content of Malmesbury sand

Property	Unit	Value	Critical level
pH	5.9		< 5 (optimal: pH 5 to pH 7)
N	Not detected		-
Calcium	cmol(+)/kg	0.42	< 1.0
Magnesium	cmol(+)/kg	0.07	< 0.4
Potassium	mg/kg	10	< 60
Sodium	mg/kg	7	> 250
Phosphorus	mg/kg	7	< 36
Total cations	cmol(+)/kg	0.56	-
Copper	mg/kg	0.71	< 0.3
Zinc	mg/kg	0.65	< 0.5
Manganese	mg/kg	0.39	< 5
Boron	mg/kg	0.02	< 0.2
Carbon	%	0.07	-
Sulphur	mg/kg	2.63	< 6.0
Iron	mg/kg	10.98	-
Field capacity	ml/ 100 g-dry sand	20 ± 3.2	-

5.3.2 Growth rates

The height of the seedlings at the beginning of the N-lignins application was between 40 and 50 mm, in all five treatments (Figure 27, **Figure 28**). This was because only seedlings with a height in this range were selected and allowed to grow, prior to the application of the N-lignins and the commercial fertilizer. Seedlings treated with N-O_5-SLS started showing signs of yellowing within 3 days of the application of this N-O_5-lignin, as shown in Figure 27 below, and eventually withered after week 1. The visual appearance of the seedlings at the end of each week is shown in the appendix (Figure 33).

Figure 27: Seedlings treated with N-lignins, after 3 days; SOD, Commercial reference, SLS, Control and KRT

According to Çoban *et al.* (2016) and Machado and Serralheiro (2017), yellowing is indicative of water, salt, or temperature stress. Since the control did not show any signs of yellowing, it is less likely that the yellowing in seedlings treated with N-O_5-SLS was caused by water or temperature stress. This effect was thus attributed to salt stress. The salt index of this material (63.62%) was nearly four times higher than that of N-O_5-KRT (16.53%) and about six times higher than that of N-O_5-SOD lignin (10.32%) (Table 16). This also corresponded to a remarkably high sodium content (144 900 mg/kg) in SLS compared to KRT (62 mg/kg) and SOD (66 mg/kg) (Table 11). The high pH (pH 8.81) of this raw material may have also had a detrimental effect on the nutrient uptake of the seedlings (Table 16).

A one-way ANOVA revealed that there was a statistically significant difference in the heights of seedlings, at the end of the cultivation period, between the 4 treatments (excluding N-O_5-SLS) (F (3, 8) = 36.81, $p < 0.001$). Post-hoc analyses (Bonferroni corrected) revealed that the heights of seedlings treated with N-O_5-KRT (107.82 mm, $p < 0.001$), N-O_5-SOD (102.72 mm, $p < 0.001$), and the commercial fertilizer (117.68 mm, $p < 0.001$) were significantly higher than the height of the control with no fertilizer (83.98 mm, $p < 0.001$) at the end of the cultivation period (Figure 28). The application of N-O_5-KRT and N-O_5-SOD thus improved the growth rates of seedlings compared to the control. This shows that these materials did not have growth inhibiting effects on the seedlings. The commercial fertilizer had a faster growth rate in week 1, due to the availability of large quantities of ammonium nitrogen, but the growth rate decreased from week 2 to week 4. N-O_5-KRT (107.82 mm, $p < 0.001$) and N-O_5-SOD (102.72 mm, $p < 0.001$) had similar growth rates, and their heights did not differ significantly at the end of week 4 ($p = 0.9598$). This could be attributed to the similarity in their ammonium nitrogen contents (Table 18).

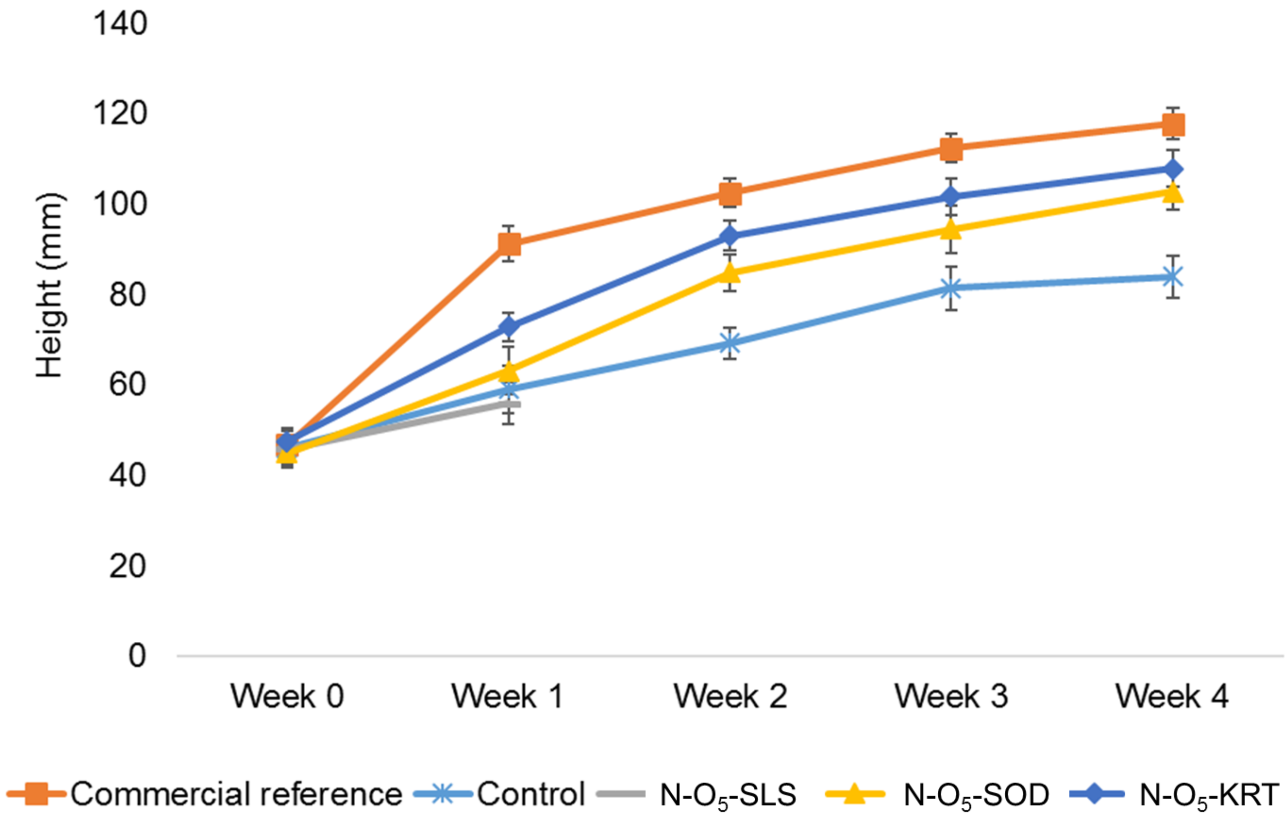


Figure 28 : Growth rate (height) of white mustard seed fertilized with N-O_5-lignins during a 4-week cultivation period

The diameters of the plants from the N-O_5-lignin treatments and the commercial fertilizer (1.82 mm, $p = 0.3046$), N-O_5-KRT (1.78 mm, $p = 0.4216$), and N-O_5-SOD (1.73 mm, $p = 0.8340$) were not statistically different from the control (1.72 mm). All treatments had an average diameter of 1.05 mm in week 0 and grew to an average diameter of 1.7 mm after week 4, as shown in Figure 29.

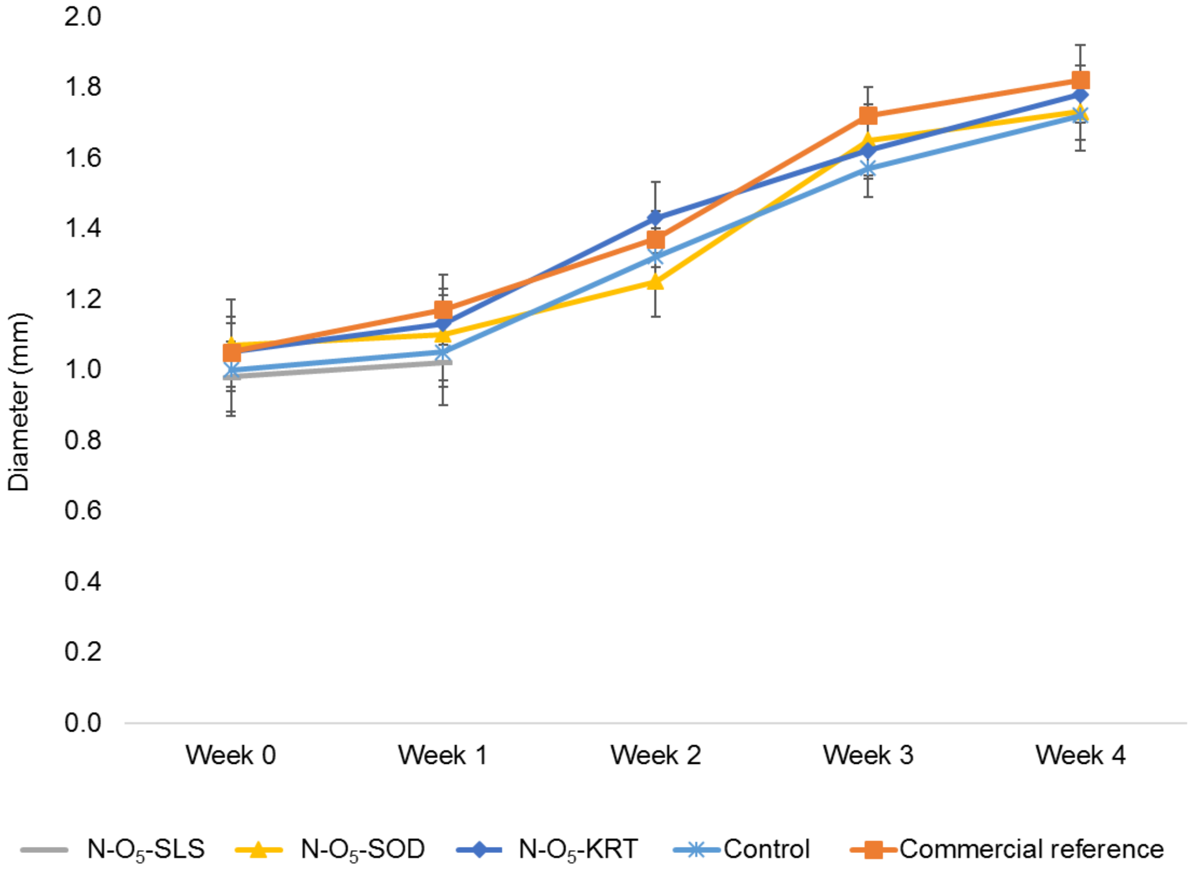


Figure 29 : Growth rate (diameter) of white mustard seed fertilized with N-O_5-lignins during a 4-week cultivation period

5.3.3 Fresh mass yield

The fresh mass yields obtained from all five treatments at the end of the cultivation period are shown in Figure 30. A one-way ANOVA revealed that there was a statistically significant difference in the fresh mass yields of the seedlings between the 5 treatments ($F (4, 10) = 144.54$, $p < 0.001$). Post-hoc analyses revealed that N-O_5-KRT (3.5 ± 0.3 t/ha, $p < 0.01$) and N-O_5-SOD (3.21 ± 0.22 t/ha, $p < 0.01$) significantly increased the fresh mass yield of seedlings by 71.6% and 57.4%, respectively (Figure 30). This improvement corresponded to the increase observed with the heights of seedlings treated with N-O_5-KRT and N-O_5-SOD (Figure 28). There was no statistically significant difference ($p = 0.2289$) between the yields obtained with these treatments (N-O_5-KRT and N-O_5-SOD) (Figure 30). The yields obtained with these N-lignins were equal to 78% and 85% of the yield obtained with the commercial fertilizer, respectively (Figure 30). N-lignins are generally expected to have lower yields compared to commercial fertilizers due to their lower nitrogen content, and the slow release of organically bound nitrogen (Ramírez *et al.*, 1997; Ramírez-Cano *et al.*, 2001). For example, Meier *et al*. (1994) reported that the yield obtained from N-lignin fertilized sorghum was 82% of the yield obtained from ammonium sulphate fertilized sorghum. The yield obtained with N-O_5-SLS (0.1 t/ha) was from the seedling remains and was not a representation of fresh plant mass because the seedlings withered after week 1 (Figure 30).

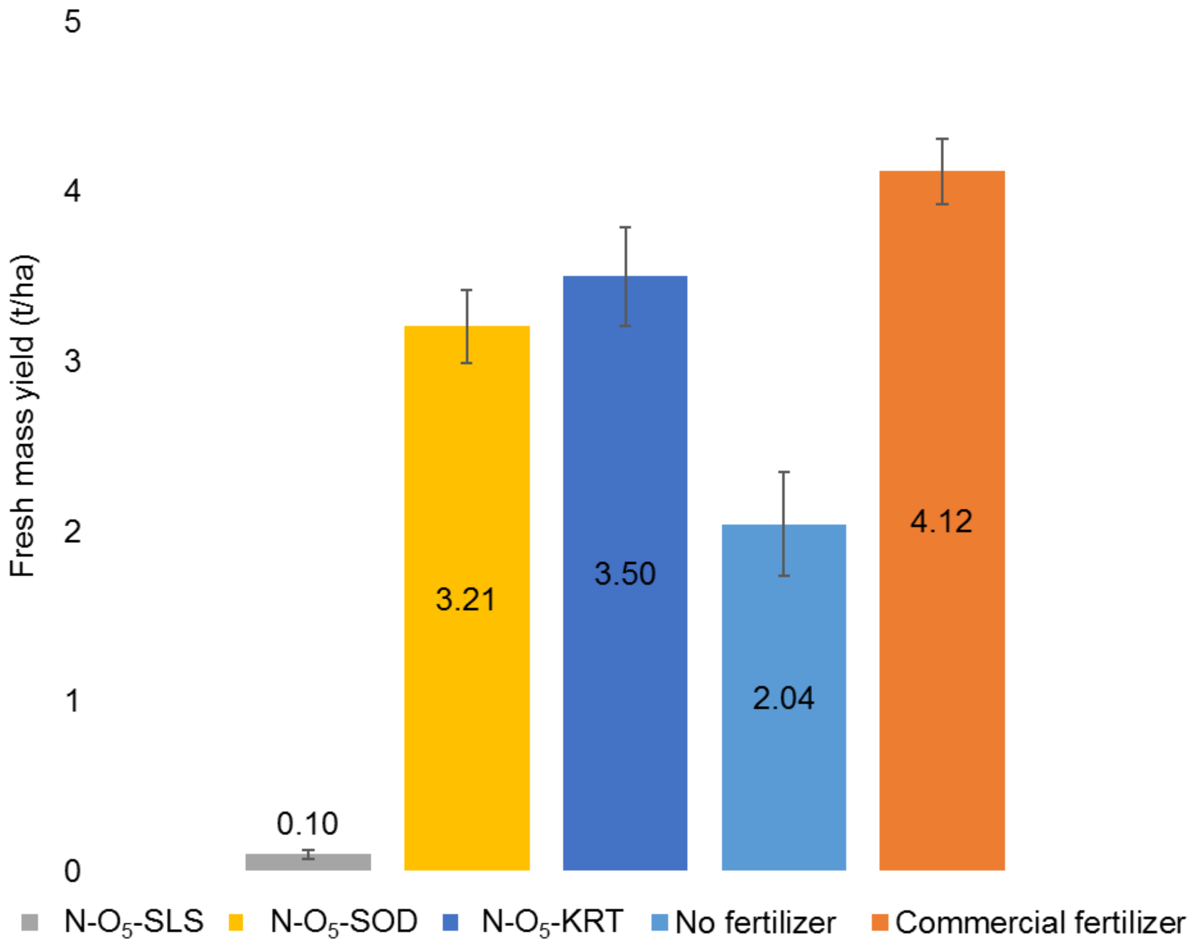


Figure 30 : Fresh mass yield of white mustard seed fertilized with N-lignin after a 4-week cultivation period

The improvement in fresh mass accumulation in seedlings treated with N-O_5-KRT, N-O_5-SOD, and the commercial fertilizer (compared to the control with no fertilizer) could also be perceived from the visual appearance of the seedlings. Seedlings from these treatments had bigger leaves, as shown in Figure 31 and Figure 32.

Figure 31: Comparison of leaf sizes, 4 weeks after fertilization; No fertilizer, Commercial fertilizer, N-O$_5$-KRT, and N-O$_5$-SOD

Figure 32 : White mustard seedlings fertilized with ammoxidized lignins, 4 weeks after fertilization; No fertilizer, Commercial reference, N-O$_5$-KRT, N-O$_5$-SOD and N-O$_5$-SLS

5.3.4 Protein mass yield

The protein mass yields obtained with N-O$_5$-KRT (227.52 ± 15.60 kg/ha) and N-O$_5$-SOD (204.95 ± 13.32 kg/ha) were significantly higher (N-O$_5$-KRT: $p < 0.001$, N-O$_5$-SOD: $p < 0.001$) than the control with no fertilizer (28.16 ± 4.60 kg/ha), while the yield obtained with N-O$_5$-SLS seedling remains (5.44 ± 3.70 kg/ha) was significantly lower ($p < 0.01$), as anticipated (Figure 33). This was consistent with findings from the fresh mass yields (Figure 30). The higher protein yield obtained with N-O$_5$-KRT (227.52 ± 15.60 kg/ha) and N-O$_5$-SOD (204.95 ± 13.32 kg/ha) in comparison to the control with no fertilizer (28.16 ± 4.60 kg/ha) indicated that some of the nitrogen bound onto these materials was absorbed by seedlings during the cultivation period (Figure 33). The protein mass yield obtained with the commercial fertilizer (277.5 ± 19.770kg/ha) was nearly 10 times higher than that of the control (28.16 ± 4.60 kg/ha). This was due to a higher fresh mass yield and a higher nitrogen accumulation in the seedlings, compared to the control (Figure 30).

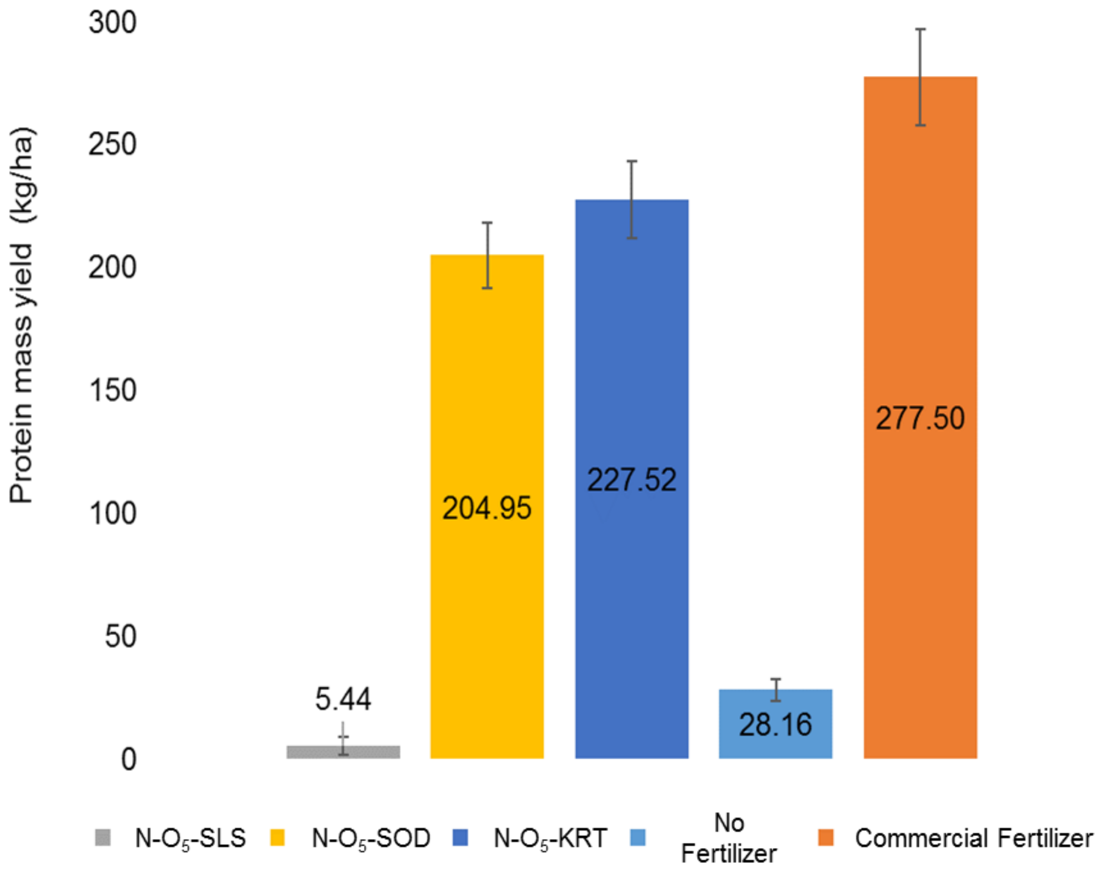


Figure 33: Protein mass yield of white mustard seed fertilized with N-lignin after a 4-week cultivation period

5.3.5 Nutrient use efficiency and Nitrogen uptake

The nutrient use efficiency (NUE) expresses the difference between the nitrogen accumulation in the seedlings from each treatment and the control as a percentage of the nitrogen applied, as shown in Figure 34. N-O_5-SLS had a NUE of -2.6%, indicating that the nitrogen accumulation was low compared to the control (Figure 34). This was consistent with the low fresh mass yield and protein mass yield of this N-lignin (Figure 33, Figure 30). N-O_5-KRT, N-O_5-SOD, and the commercial fertilizer improved the nitrogen accumulation in the seedlings compared to the control, having NUE of 8.97%, 9.57% and 16.62%, respectively (Figure 34). The nitrogen uptake as a percentage of nitrogen applied (NUA) was 11.12% and 10.26% for N-O_5-SOD and N-O_5-KRT, respectively (Figure 34). The NUA was slightly higher for N-O_5-SOD because similar amounts of nitrogen were accumulated in the seedlings but N-O_5-SOD had a lower nitrogen content (Table 18, Figure 34). Ramírez *et al*., (2007) found that the NUA of N-lignin in pumpkins (*Zucchini cucurbita pepo L*) was higher at 16.73%. This was due to the use of a longer cultivation period (60 days) compared to this study (28 days).

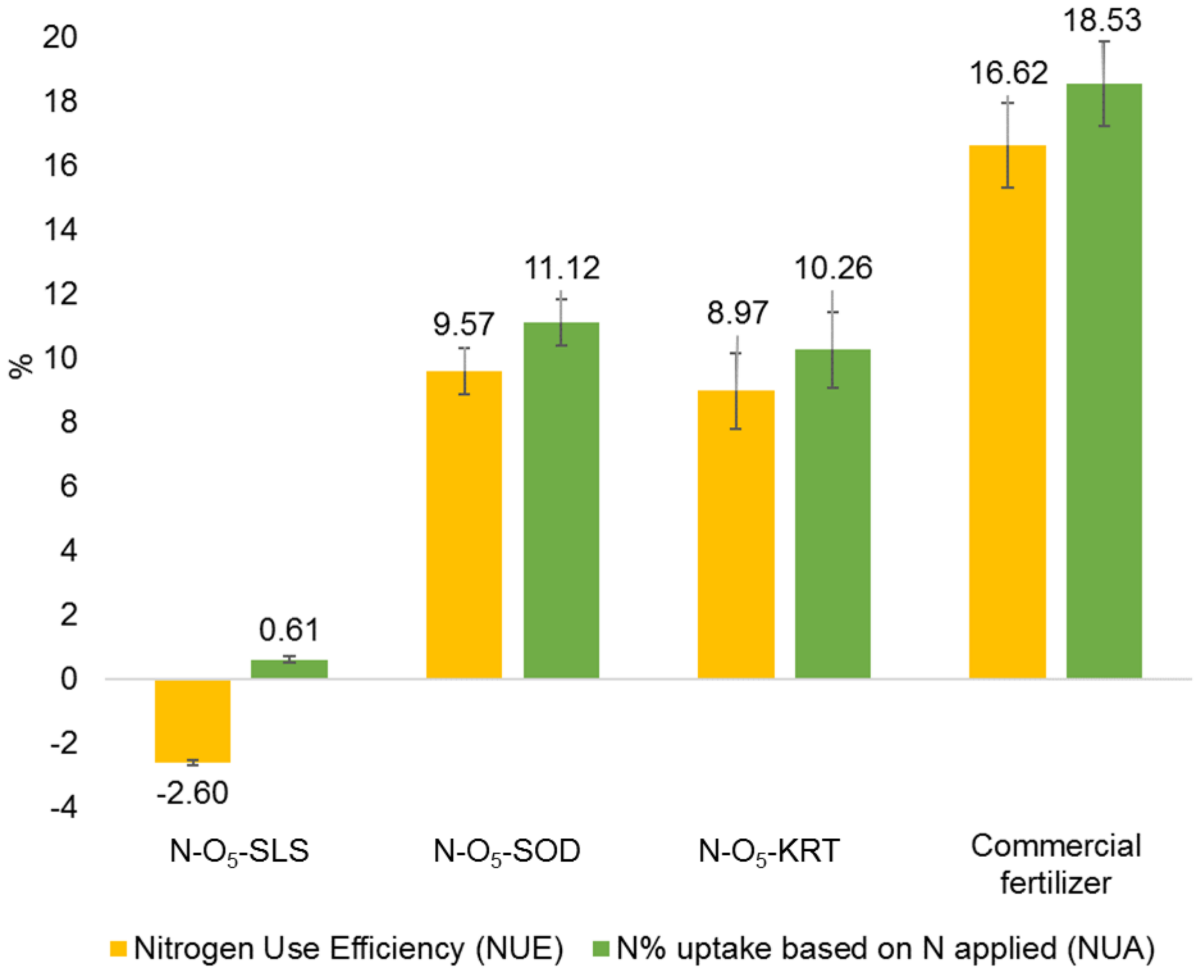


Figure 34: NUE and N% uptake of white mustard seed fertilized with N-O_5-lignin after a 4-week cultivation period

6 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

The present study demonstrated that N-lignins with C/N ratios of less than 20 could be synthesized from lignins obtained from South African pulp and paper mills using air instead of pure oxygen. With pre-oxidation, the desired C/N levels (< 20) could be achieved in all three lignins, meeting the standard for use as soil organic amendments. However, a plant trial assessment showed that only N-lignins from KRT and SOD were suitable for use as soil organic amendments.

Characterization of the raw materials through UV spectroscopy and FTIR revealed that KRT had a considerably higher amount of phenolic hydroxyl groups compared to SOD and SLS. This indicated that KRT would be more reactive to oxidation compared to SOD and SLS. Since oxidation is the rate determining step in ammoxidation, and it is known that ~ 60% of the nitrogen incorporated in N-lignins is related to degradation of phenolic structures, it could be predicted that KRT would incorporate more nitrogen during ammoxidation. FTIR spectroscopy revealed that KRT and SOD contained a higher abundance of methoxyl groups in their chemical structures compared to SLS. This was attributed to the biomass origin of these lignins (KRT: hardwood, SOD: bagasse, and SLS: mixture of hardwood and softwood). SLS had a considerably high ash content, particularly sodium, originating from the pulping chemicals. This made this raw material suspect to causing toxicity to plants and causing pH increases in the soil upon the dissolution of the sodium. All three raw materials contained plant nutrients such as phosphorus and potassium, which bodes well for their use as soil amendments.

Upon ammoxidation, the reactivity of these lignins could be ranked in the following order: KRT > SOD > SLS, confirming the postulations made based on the raw material properties. KRT could be ammoxidized to reach the desired C/N levels (<20) without pre-oxidation. SOD and SLS required pre-oxidation with 5% H_2O_2 to achieve C/N ratio less than 20 after ammoxidation. However, the N-lignins obtained in this study had lower nitrogen contents and higher proportions of inorganically bound nitrogen compared to N-lignins reported in literature, due to a lower extent of oxidation achieved in the reaction setup using air as the oxidant instead of oxygen, and a lower quantity of oxygen-rich functional groups compared to lignins used in previous studies.

Seedlings treated with N-O_5-SLS withered within 3 days of the application of this material, attributed to the high salt index and high pH of the material compared to N-O_5-KRT and N-O_5-SOD. The pH levels of N-O_5-KRT and N-O_5-SOD were within the range for optimum plant growth, and these materials had low salt indexes. Seedlings treated with N-O_5-KRT and N-O_5-SOD had higher fresh mass and protein mass yields compared to the control, indicating that these materials did not exhibit growth inhibition effects, and that the seedlings were able to absorb nitrogen contained in these materials as evidenced by an increased protein yield.

This study showed that all three raw materials could be used to synthesize N-lignins with desirable C/N despite the difference in their reactivity due to biomass origin and isolation method. However, the high ash content of SLS, especially sodium, limited its use as a soil organic amendment by imparting undesirable properties to the material such as a high pH upon dissolution, and a high salt index.

6.2 Recommendations

The following is a list of recommendations made to improve the use of South African technical lignins for use as raw materials for ammoxidation, and to improve the knowledge and understanding of the effect of N-lignins in soils;

- SLS could be pre-oxidized and ammoxidized to produce an N-lignin product with a desirable C/N ratio. However, the high sodium content in the material made it unsuitable for use as an organic amendment. This element cannot be completely removed, because it originates from the pulping reagents used in the production of this raw material. However, ultrafiltration may be used to decrease its content in the material below toxic levels. The carbohydrate content may also be reduced if this step is combined with acid digestion (Humpert *et al.*, 2019). This route may be expensive for industrial production. However, it may provide new insights into the reactivity of SLS to ammoxidation. Ultrafiltration may also reduce the content of chemical species such as sodium sulphite which will compete with the material for oxidants. The high pH of the material may be a desirable property for use as a soil amendment in strongly acidic soils.
- The ammoxidation of KRT and SOD (with pre-oxidation) yielded N-lignins with sufficiently low C/N ratios, and the application of these materials improved the growth of seedlings. Process conditions may be optimised for the ammoxidation

of each lignin type and a techno-economic assessment could be conducted for their industrial production to assess the profitability of this process. Beckman and Radlein (2000) showed that the industrial production of nitrogenous fertilizers through ammoxidation of bio-oil is economically feasible.

- The N-lignins synthesized in this study had low total nitrogen contents, and high NH_4-N contents compared to the N-lignins in literature. The effect of stronger oxidizing agents such as Fenton reagents could be investigated to improve nitrogen incorporation.
- Further studies can investigate whether N-lignins are suitable for use as mixtures with mineral fertilizers. The possibility of using such mixtures can strike the balance between improving crop yields for the short term, and improving SOM contents for long term productivity.
- Long term studies on soil rehabilitation with N-lignins can provide insights as to how the continued application of N-lignins affects soil microbial activity and plant growth, and will evaluate whether the long-term application of N-lignins leads to the accumulation of plant toxic minerals that originate from pulping chemicals.
- In this study, the N-lignins were tested on a single soil type, and a single plant species over a four-week cultivation period. Although the results of the trial could provide some insights into the suitability of these materials, medium term plant trials with different plant species and soil types (acidic, alkaline, loamy, clayey) are necessary to assess whether the N-lignins are mineralized completely in the soil to release nitrogen and form humic substances, and whether they have the expected effect on plant growth, and soil humus levels.

Despite areas of further research into the use of N-lignins as soil organic amendments, the performance of N-lignins in plant trials as shown in the literature and in this study, indicates that these products could potentially be produced at industrial scale and used to improve SOM and sequester atmospheric carbon dioxide. This would be in support of the re-carbonization of global soils initiative, launched by the Food and Agriculture organization (Rickson *et al.*, 2015; Amelung *et al.*, 2020).

“*The challenge is clear. The world must produce 40% more food, with limited land and water, using less energy, fertilizer and pesticide—by 2030*”

(Beddington, 2010)

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8 APPENDICES

Table 20: Chemical composition of Wonder^{TM} all-purpose plant food

Nutrient	Concentration
Nitrogen	81.45 g/ℓ
Phosphorus	17 g/ℓ
Potassium	48 g/ℓ
Magnesium	1.11 mg/ℓ
Sulphur	0.32 mg/ℓ
Iron	23.01 mg/ℓ
Manganese	43.05 mg/ℓ
Zinc	124.16 mg/ℓ
Copper	27.81 mg/ℓ
Boron	422.19 mg/ℓ
Molybdenum	261.59 mg/ℓ
Specific gravity	1.21

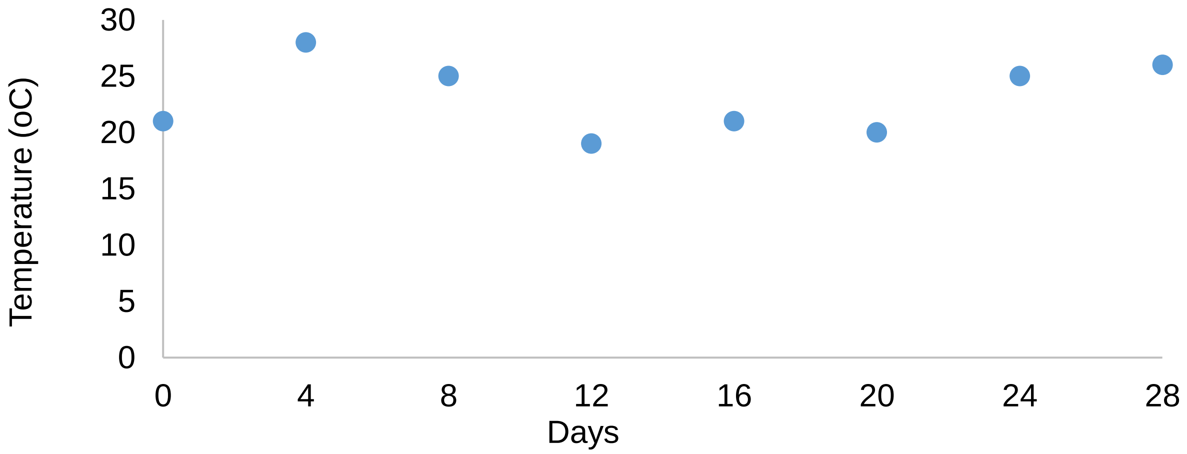

Figure 35: Temperature variation in the nursery over the growth period

8.1 Plant trial

Figure 36: White mustard seedlings fertilized with pre-oxidized and ammoxidized lignins over a 4-week cultivation period