POLITECNICO DI TORINO

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Evaluation of solvent-catalyst systems for biphasic depolymerization of Kraft lignin

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1 Riassunto

1.1 Introduzione

Oggigiorno, i problemi climatici e l'esaurimento delle risorse fossili rappresentano un problema che in ambito scientifico si sta cercando di risolvere, tentando di trovare vie alternative all'attuale economia dell'industria petrolifera. Il problema non ricade unicamente nel trovare valevoli opzioni per la produzione di energia ma anche nel trovare metodi alternativi per la produzione degli attuali prodotti chimici derivanti dalle raffinerie: ben il %5 dell'*oil&gas* prodotti oggigiorno è destinato all'industria petrolchimica. È, quindi, necessario trovare valide opzioni per progettare bioraffinerie di seconda generazione per il trattamento di biomasse. La più abbondante biomassa presente al mondo che non compete con il settore alimentare è la lignocellulosa. Il progetto sviluppato in questa tesi si colloca in questo ambito, ovvero nella ricerca di valevoli opzioni per produrre sostenibilmente prodotti chimici a partire dalla lignina.
La biomassa lignocellulosica è stata identificata come la più abbondante biomassa sulla terra ed è composta prevalentemente da tre biopolimeri: cellulosa (40-60%), emicellulosa (10-40%) e lignina (15-30%) [1, 2, 3, 4]. La composizione della biomassa varia in funzione della specie e dall'età della pianta, nonché anche dalle possibili condizioni climatiche in cui la pianta sia cresciuta [1]. La cellulosa e la emicellulosa sono dei polisaccaridi, invece la lignina è un biopolimero amorfo caratterizzato da un'elevata eterogeneità e irregolarità strutturale. Data la complessità della lignina, per la sua caratterizzazione si usa fare riferimento alle unità fondamentali tipiche della lignina ovvero alcol cumarilico (4-[(E)-3-Idrossiprop-1-enil]fenolo) (H), alcol coniferilico (alcol 4-idrossi-3-metossicinnamilico) (G), e alcol sinapilico (alcol 4-idrossi-3,5-dimetossicinnamilico) (S). Altro aspetto da dover considerare nella caratterizzazione della lignina è la tipologia di legami presenti che permettono di mantenere unite le differenti unità monomeriche: il legame più abbondante è il legame β-O-4, ma sono anche presenti legami C-C , i quali sono estremamente recalcitranti alla rottura [1, 5].
Le caratteristiche della lignina sono anche fortemente influenzate dalla metodologia di estrazione della lignina dalla biomassa lignocellulosica. Dato che la frazione di carboidrati presenti nella lignocellulosa ha trovato largo impiego nell'industria, gli attuali processi industriali per l'estrazione dei biopolimeri dalla lignocellulosa non presentano cura nel cercare di mantenere l'integrità strutturale della lignina il più i simile alla struttura nativa. Svariati processi per il frazionamento della biomassa lignocellulosica esistono, come ad esempio il processo Kraft, processo Soda, processo al solfito o il processo Organosolv. Il processo industriale più ampiamente utilizzato è il processo Kraft che produce annualmente tra le 50-55 tonnellate di lignina Kraft all'anno [6]. Questa tecnica di frazionamento prevede la digestione della biomassa lignocellulosica a temperature moderate (145-170°C) in presenza di idrossido di sodio (NaOH) e solfuro di sodio (Na_2S). L'elevato pH permette la solubilizzazione della lignina e la delignificazione della biomassa. Allo stesso tempo però, l'elevato pH promuove la rottura dei legami β-O-4 della lignina causando una condensazione e ripolimerizzazione della lignina[1, 7]. La frazione contenete i carboidrati è divisa dalla lignina e si ottiene una soluzione chiamata “black liquor” in cui è disciolta la lignina estratta. Solitamente la lignina è isolata da questa soluzione promuovendo la sua precipitazione mediante acidificazione [8, 6]. Per la trattazione degli altri metodi di estrazione si rimanda alla sezione in inglese 2.2.
La ricerca di vari metodi per la valorizzazione della lignina ha trovato grande interesse

e attenzione per trovare una soluzione su come integrare la lignina nel concetto di bioraffineria. La depolimerizzazione della lignina è stata studiata per vari tipi di lignina sia con catalizzatori omogenei che eterogenei. Una generale classificazione dei metodi di depolimerizzazione della lignina li divide in cinque categorie: (i) depolimerizzazione in presenza di catalizzatori acidi/basici, (ii) riduttivi, (iii) ossidativi, (iv) depolimerizzazione solvolitica e (v) trattamenti termici. Nella sezione 2.4 è fornita una generale descrizione di questi vari metodi.

1.2 Obiettivi

Nel concetto di bioraffineria di seconda generazione, in cui la biomassa trattata non deve competere con il settore alimentare, l'ideale sarebbe di poter valorizzare al meglio ogni componente della biomassa. Nel trattamento della biomassa lignocellulosica la frazione di carboidrati può essere facilmente idrolizzata in zuccheri e quindi trasformati, ad esempio, in etanolo attraverso un processo di fermentazione. La lignina estratta dalla biomassa è tendenzialmente sottoposta a combustione, ma con scarso recupero energetico dato il suo basso potere calorifico. In questa tesi si è cercato di progettare un sistema per la valorizzazione della lignina Kraft in composti aromatici. Questo progetto di ricerca è stato svolto presso il centro di ricerca *Paul Scherrer Institut* (PSI), Svizzera nel progetto *SolvoLig2Chem* del gruppo di ricerca *Catalytic Process Engineering.*

In particolare, l'idea è di designare un sistema bifasico per la depolimerizzazione della lignina: l'ambiente di reazione è costituito da una fase polare acquosa in cui è disciolto il catalizzatore e una fase apolare il cui scopo è di garantire il recupero dei prodotti di reazione, proteggendoli così da reazioni di condensazione e ripolimerizzazione (reazioni accertate come altamente probabili durante la depolimerizzazione). Un articolo già pubblicato da questo gruppo di ricerca ha mostrato le potenziali di questa idea: è stato investigato un sistema bifasico acqua-ottanolo in presenza di acido solforico come catalizzatore utilizzando lignina Kraft come materiale di partenza. In questo studio, hanno dimostrato come il sistema bifasico permetta una resa di vanillina come prodotto di reazione sei volte maggiore rispetto al sistema in presenza della sola fase acquosa [9]. Nel progetto qui presentato si è cercato di ideare un nuovo sistema bifasico per il trattamento della lignina in presenza di poliossometalati (POMs) come catalizzatori, i quali agiscono come agenti ossidanti stechiometrici in assenza di O_2.

1.3 Materiali, Metodi e Strumentazione

Una lista completa di tutte le sostanze chimiche utilizzate è elencata in Tab. 3.1. La tipologia di lignina utilizzata per tutti gli esperimenti è una lignina dolce di tipo Kraft Indulin AT (in inglese: *Softwood Kraft Lignin Indulin AT*) fornita da MeadWestvaco, il cui contenuto di acqua si è considerato essere 3.5% wt.

Le proprietà della lignina sono fortemente influenzate dalla metodologia di estrazione utilizzata. La lignina dolce Kraft è un biopolimero caratterizzato da un elevato peso medio molecolare ponderale ($M_w = 4290$) e da un elevato indice di polidispersità ($PDI = 8.1$) [10]. La lignina è solubile in ambiente basico e quasi totalmente insolubile in ambiente acido a temperatura ambiente, ed aumenta la sua solubilità all'aumentare della temperatura [1, 10]. Un grande svantaggio della lignina Kraft è la presenza di residui di zolfo dal processo di frazionamento, il quale è noto per essere velenoso per moltissimi catalizzatori. Inoltre, su 100 unità aromatiche questa tipologia di lignina ha 97 unità G, 3

H e nessuna unità S è stata identificata e 6.1 legami β-O-4 [10]. I legami β-O4 sono i legami che solitamente si cercano di rompere durante la depolimerizzazione della lignina, in quanto i legami C-C sono molto più difficili da rompere: è da notare che la lignina dolce di tipo nativo è caratterizzata da 45 legami $\beta - O - 4$ su 100 unità aromatiche, e quindi è abbastanza palese come il processo di estrazione abbia influenzato le caratteristiche della lignina e quindi le sue potenzialità nella sua valorizzazione.
La tipologia di reattore utilizzato è un Microwave Bacth reactor Biotage® Initiator+. Questo reattore permette di preparare l'ambiente di reazione in dei vials di vetro da 10 ml i quali vengono inseriti successivamente nel reattore: attraverso un'interfaccia grafica è possibili impostare la temperatura, il tempo di reazione e la velocità di agitazione. Inoltre, è possibile monitorare durante il tempo di reazione l'andamento della temperatura, della pressione e della potenza fornita. Per maggiori dettagli in merito alla modalità di preparazione degli esperimenti e sul reattore è possibile consultare le sezioni 3.3 e 3.4. Alla fine del tempo di reazione, le due fasi presenti sono recuperate mediante una siringa, filtrate e quindi conservate in appositi contenitori. L'analisi dei prodotti di reazione è stata effettuata usando uno UHPLC-HRMS: una curva di calibrazione per la vanillina e una per la siringaldeide sono state effettuate usando come standard interno un isotopo della vanillina caratterizzato dalla presenza di ^{13}C nell'anello aromatico ($^{13}C_6H_8{}^{12}C_2O_3$). Le due curve ottenute sono caratterizzate entrambe da un $R^2 = 0.999$ e le equazioni sono le seguenti:

$$Vanillina : y = 0.9778x \tag{1.1}$$

$$Sinringaldeide : y = 1.1854x \tag{1.2}$$

dove y rappresenta il rapporto tra l'area di vanillina o siringaldeide e quella dello standard interno, mentre x rappresenta il rapporto tra la concentrazione di vanillina o siringaldeide e la concentrazione di standard interno. Le due rette di calibrazione sono state costruite in un range tra 100-0.1 μM, verificando la validità della retta trovata analizzando per entrambe le rette tre *Quality Control Samples (QCs).* I risultati ottenuti attraverso l' UHPLC-HRMS sono stati analizzati *in primis* attraverso il software Thermo Scientic^{TM} Compound Discover^{TM} 3.0: questo software permette di identificare le sostanze presenti nei campioni analizzati mediante comparazione con librerie ampiamente affermate (mzCloud^{TM} e ChemSpider). Quindi, dopo aver selezionato i composti da analizzare, si effettua l'analisi dei risultatati ottenuti sviluppando un metodo di analisi mediante il software Thermo Fischer Xcalibur$^{4.2}$: questo software permette di ottenere dei file CSV in cui è possibile leggere i valori delle aree dei composti desiderati e dello standard interno misurati. Tutti i risultati presentati in questa tesi sono stati ripetuti tre volte al fine di garantire una riproducibilità e attendibilità dei dati forniti. Al fine di velocizzare il trattamento dei dati è stata sviluppato un codice Matlab, i cui dettagli sono spiegati nella sezione 3.6.1 e il codice è presente in Appendice B.

1.4 Risultati

In questo studio sono stati variati diversi parametri al fine di indagare le potenzialità di un sistema bifasico per la depolimerizzazione in presenza di POMs, ponendo particolare attenzione a come siano influenzate al variare dei parametri operativi la resa di reazione, la selettività, la solubilità e stabilità dei prodotti.

I poliossometalati (POMs) possono essere definiti dei cluster polianionici costituiti principalmente da metalli di transizione. Sono forti acidi di Brønsted e Lewis. Si è dimostrato che questi composti permettono l'ossidazione della lignina e possono essere facilmente riossidati in presenza di ossigeno, come mostrato dalle seguenti reazioni [11]:

$$POM_{(ox)} + Lignina \rightarrow POM_{(red)} + Lignina_{(ox)} \tag{1.3}$$

$$POM_{(red)} + O_2 \rightarrow POM_{(ox)} + H_2O \tag{1.4}$$

Queste due reazioni possono accadere solo se:

$$^{\circ}E_{Lignina} > {}^{\circ}E_{POM} > {}^{\circ}E_{O_2} \tag{1.5}$$

dove $^{\circ}E_{\mathrm{O}_2}$, $^{\circ}E_{\mathrm{POM}}$,$^{\circ}E_{\mathrm{O}_2}$ sono rispettivamente i potenziali ossidativi della lignina, dei POMs e dell'O_2. è stato possibile trovare in letteratura i valori dei potenziali ossidativi: per la lignina è pari a 0.4-0.6 eV vs. NHE a pH pari ad 1 [11, 12, 13], per i POMs varia tra 0.68-0.71 eV vs. NHE a pH pari ad 1 [11, 13, 14], e per ossigeno è pari a 1.22 eV vs. NHE a pH pari a 0.059 [11].

Il primo passo effettuato per la progettazione del sistema bifasico è stato la selezione del solvente organico, il quale è stato scelto valutando: solubilità dei prodotti di reazione, stabilità e greenness del solvente. Sono stati comparati quattro diversi sistemi in presenza di 1 mg/ml di lignina Kraft e una concentrazione di POM-Mo 10 μM in presenza di argon e con un tempo di reazione pari a 20 min a 170°C. I vari sistemi analizzati sono mostrati in Tab.1.1

Solventi	**Catalizzatore [μM]**	**Gas**	**Temperatura [°C]**
Acqua - Ottanolo	POM-Mo (10)	Ar	170
Acqua - Toluene	POM-Mo (10)	Ar	170
Acqua Salata[a] - Acetonitrile	POM-Mo (10)	Ar	170
Acqua Salata[a]- Tert-Butanolo	POM-Mo (10)	Ar	170

[a] Salty water means Milli-Q water saturated with NaCl.

Table 1.1: Lista dei sistemi bifasici analizzati. Ogni esperimento è stato condotto in presenza di 1 mg/ml di ligna Kraft per un tempo di reazione di 20 min.

Il solvente che si è dimostrato il miglior candidato è stato il toluene, il quale, in particolare, ha mostrato di essere inerte dalla presenza del POM-Mo e quindi di garantire stabilità ai prodotti di reazione. Tuttavia, il toluene è stato il solvente ha mostrare il minor valore di solubilità della vanillina rispetto agli altri solventi presi in analisi.

Dopo la scelta del toluene come fase organica del sistema bifasico, si è studiato l'attività di due poliossometalati, acido fosfomolibdico (POM-Mo) e acido tungstosilicico (POM-W) al variare della loro concentrazione in un range tra 1 e 100 mM. Inoltre, sono stati effettuati degli esperimenti monofasici in presenza della stessa concentrazione di catalizzatore per poter capire se anche in questo caso il sistema bifasico porti a dei vantaggi. Dato il carattere acido sia del sia di POM-Mo che di POM-W, si sono effettuati esperimenti in presenza di H_2SO_4, per comprendere se l'attività catalitica sia dovuta al carattere acido del catalizzatore o dalla sua capacità di agire come agente ossidante. Come parametro per questo *screening* iniziale, si è usato la resa di vanillina, la quale si è dimostrata essere il principale prodotto di reazione. I risultati sono sintetizzati nella Fig.1.1

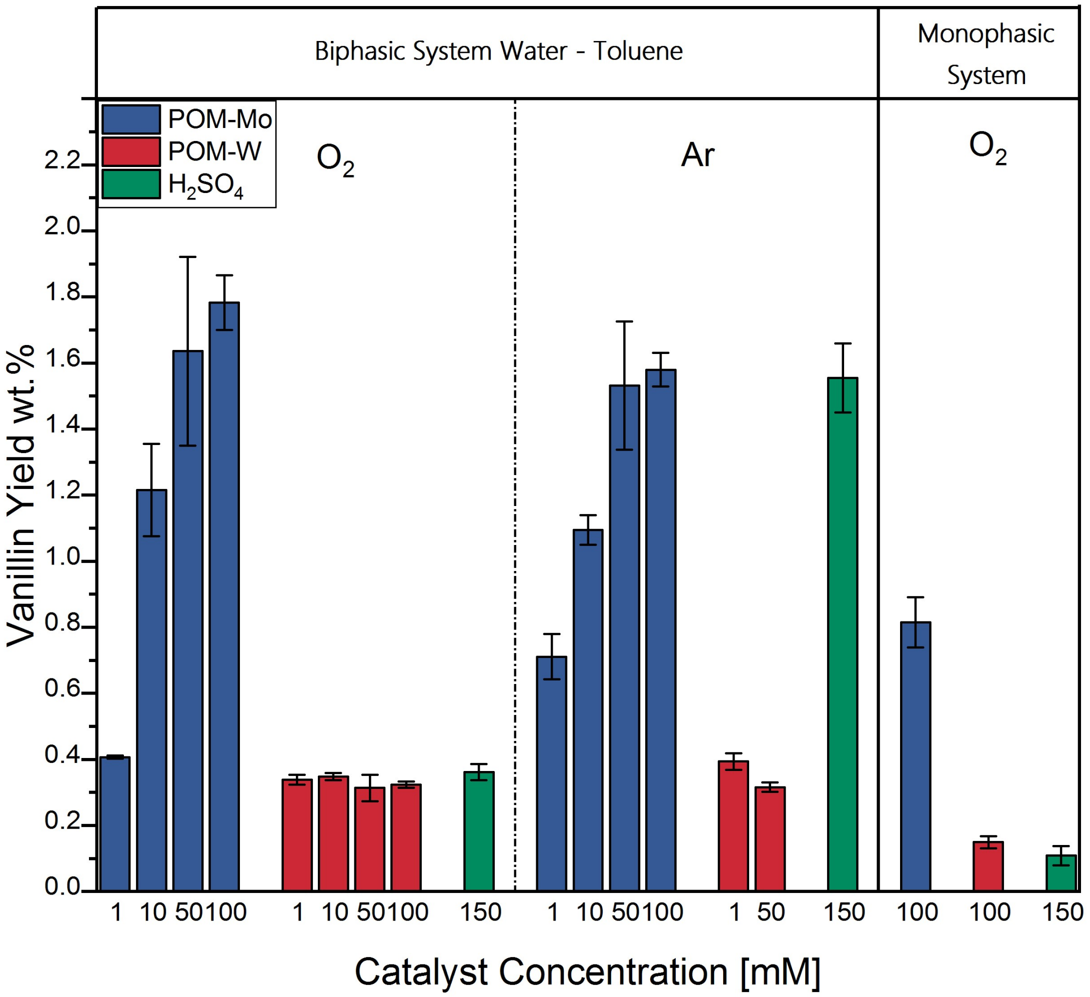


Figure 1.1: Rappresentazione della resa di vanillina di una serie di esperimenti bifasici e monofasici condotti in presenza di 1 mg/ml di lignina Kraft a 170°C per 20 min.
Asse x: concentrazione del catalizzatore (riferita alla fase polare).
Asse y: resa di vanillina in peso normalizzata rispetto alla massa di lignina.

I risultati hanno dimostrato come il sistema bifasico permetti di avere delle rese di reazione più del doppio rispetto ai corrispettivi esperimenti condotti in una soluzione acquosa. Inoltre, il POM-Mo ha permesso di ottenere delle rese di reazione nettamente maggiori rispetto al POM-W. Si può notare come la presenza dell'O_2 o dell'Ar non influenzi in modo particolare la resa di reazione, ad eccezione fatta per H_2SO_4. Il reattore utilizzato garantisce l'agitazione semplicemente attraverso un agitatore magnetico ed inoltre la presenza del toluene agisce come barriera per l'ossigeno presente. Di conseguenza, è possibile ipotizzare che per aspetti impiantisci del reattore e fluidodinamici il poco O_2 presente nell'ambiente di reazione non riesce ad interagire con il POM-Mo per effettuare la sua rigenerazione. Per tale motivo, si è deciso di continuare lo studio del sistema bifasico in

un'atmosfera protettiva di Ar.
L'idea della progettazione di un sistema bifasico si basa anche potezialità di proteggere i prodotti di reazione da possibili fenomeni di condensazione e ripolimerizzazione. Per investigare se il toluene permetesse tale fenomeno di protezione, sono stati effettuati degli esperimenti in presenza di vanillina come materiale di partenza e catalizzatore: i risultati hanno mostrato che in presenza di 50 mM di POM-Mo in un sistemano monofasico la perdita di vanillina è circa par al 14 %, invece nel sistema bifasico si è riscontrata una perdita di vanillina pari al 5%. È possibile , quindi, affermare che la presenza del toluene permetta la protezione della vanillina nel decorso del tempo di reazione.
Scelto il catalizzatore POM-Mo, la concentrazione con cui desiderare continuare lo studio e in presenza di quale gas condurre la reazione, si è desiderato studiare come la temperatura possa influenzare la resa di reazione. La temperatura è stata variata in un range tra 140 e 200 °C con un intervallo di 10 °C e la resa totale di reazione e di vanillina in funzione della temperatura sono mostrate in fig.1.2.

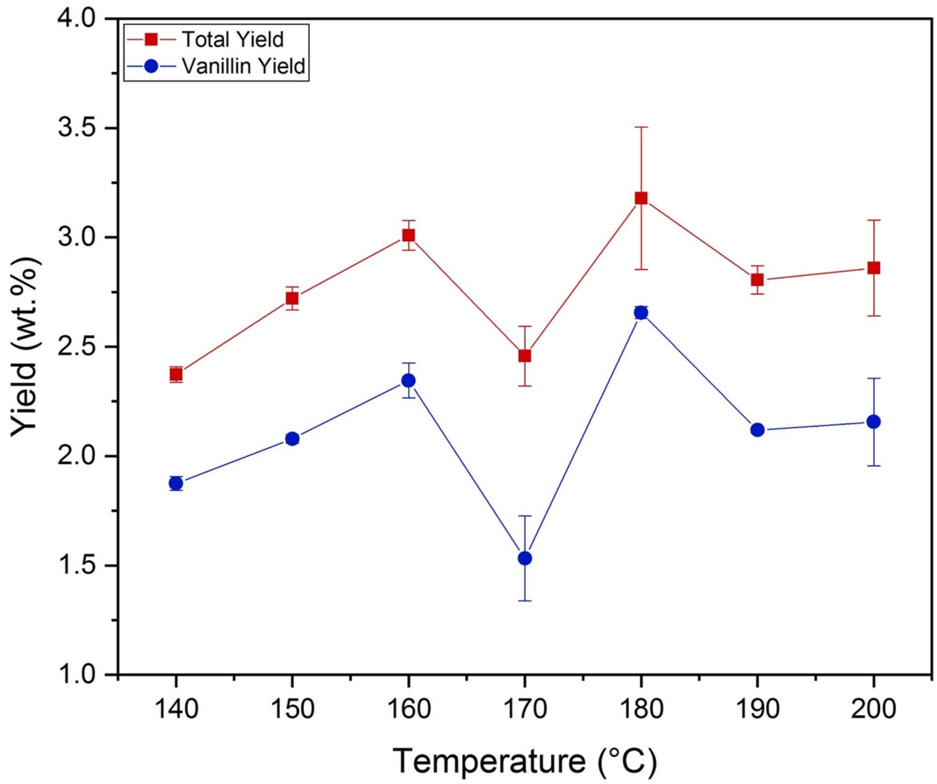
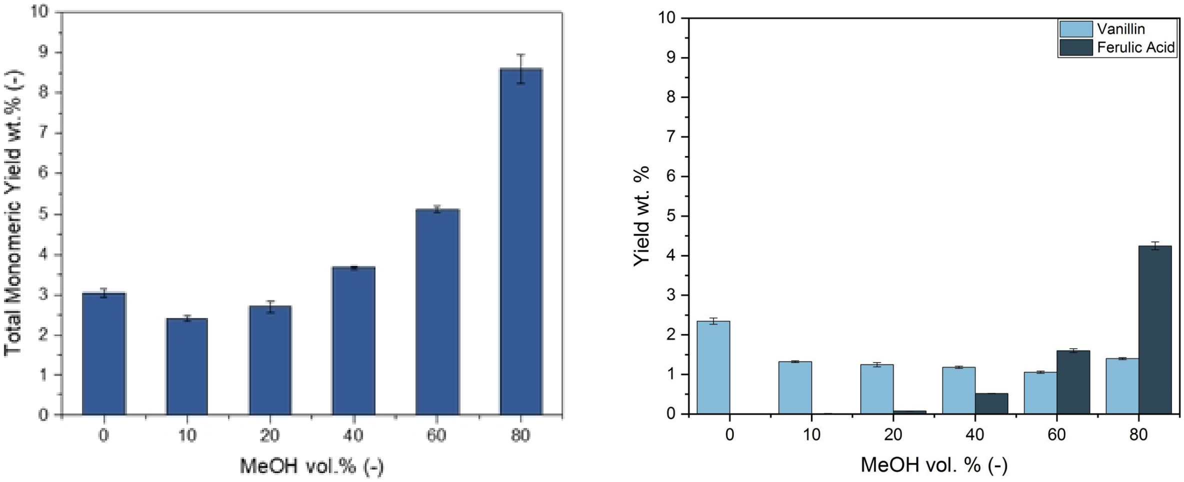


Figure 1.2: Influenza della temperatura sulla resa di reazione. In rosso la resa totale monomerica (inteso come tutti i composti identificati), in blu la resa di vanillina. Gli esperimenti sono stati condotti in un sistema acqua-toluene con 1 mg/ml di lignina Kraft in presenza di 50 mM di POM-Mo in un'atmosfera di Ar.

I risultati mostrano un andamento particolare: la resa totale e quella della vanillina aumentano all'aumentare della temperatura sino a 170°C, dove si riscontra il minimo sia per la resa totale che per la resa di vanillina nell'intervallo di temperatura analizzato. A 180°C si riscontra il massimo per entrambe le rese analizzate (resa totale 3.19wt.% e resa di vanillina pari a 2.16 wt%). Una possibile spiegazione il punto di minimo a 170°C può risiedere nelle proprietà della lignina: in letteratura è stato possibile trovare che la temperatura di transizione vetrosa della lignina Kraft è compresa tra i 160-170°C [15, 16].

Dato che la transizione di stato è un processo endotermico, è possibile ipotizzare che la lignina "sottragga" del calore disponibile al catalizzatore al fine di effettuare la transizione di stato.

La presenza del metanolo durante la depolimerizzazione della lignina sembra che permetta di effettuare una alchilazione radicalica [1]. Uno studio effettuato da un gruppo dell'ETHZ ha studiato il comportamento della depolimerizzazione della lignina dolce Kraft in presenza di POM-Mo e MeOH in ambiente acquoso: i loro risultati mostrano che la presenza di MeOH permetta di avere un incremento della resa di reazione più del doppio solo in presenza di un'atmosfera ossidativa e non inerte [17, 18]. Inoltre, hanno dimostrato come la presenza del MeOH permetta la produzione di un nuovo prodotto di reazione, ovvero il 4-idrossi-3-metossibenzoato di metile, il quale è la vanillina con un gruppo metilico in più [17, 18]. Il primo passo per investigare il ruolo del MeOH in un sistema bifasico per la depolimerizzazione della vanillina è stato di appurare se il sistema rimanesse bifasico: da prove sperimentali di laboratorio, si è visto che un sistema con una soluzione acqua - MeOH 80vol.% unita al toluene è ancora bifasico a temperatura ambiente. La lignina in presenza di MeOH ha dimostrato un decisivo incremento della sua solubilità nella fase polare. Sono state studiate varie concentrazioni di MeOH (10, 20, 40, 60, 80 vol.% rispetto alla fase polare). La temperatura di reazione scelta è stata di 160 °C, in quanto il sistema non si dimostrava stabile in presenza di 80%vol. di MeOH a 180°C, dove precedentemente si erano riscontrato i valori massimi in termini di rese.



(a) (b)

Figure 1.3: Influenza della concentrazione di MeOH su (a) resa monomerica totale and (b) resa di vanillina e acido ferulico. La percentuale in volume del MeOH è riferita al volume della fase polare. Gli esperimenti sono stati condotti a 160°C per 20 minuti con Ar e 50 mM di POM-Mo.

I risultati, riassunti in fig.1.3a e 1.3, hanno mostrato un netto aumento della resa totale monomerica di reazione all'aumentare della concentrazione di MeOH: in presenza di 80 vol.% di MeOH si è ottenuta una resa totale di 8.59 wt.% rispetto ad un 3.01wt.% in assenza di MeOH. La presenza della seconda fase sicuramente ha giocato un ruolo nell'aumentare la resa di reazione: lo studio precedentemente menzionato dell'ETHZ hanno ottenuto una resa di reazione di 5.18wt.% in presenza di 80 vol. % di MeOH e O_2 e di 1.24 wt.% in presenza di 80 vol. % di MeOH e N_2. Inoltre, una sostanziale dif-

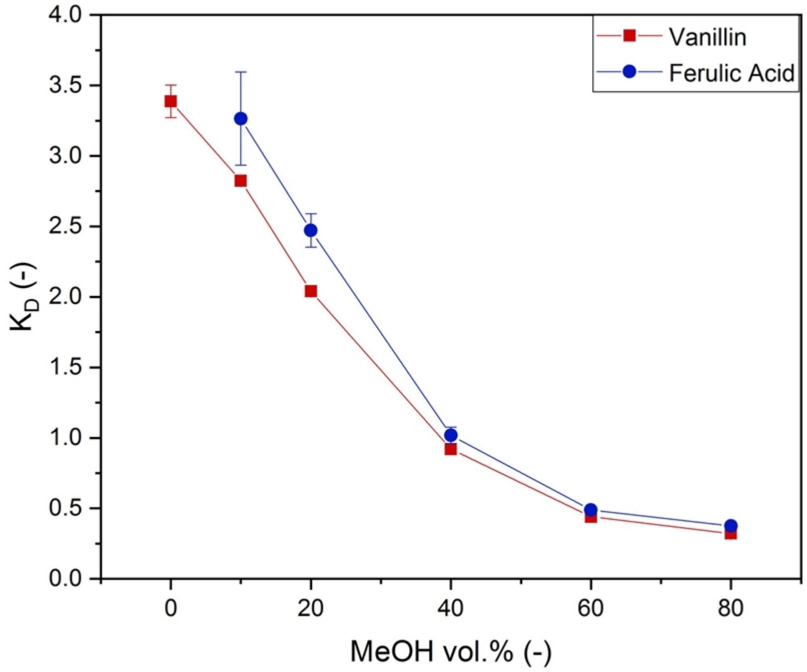


Figure 1.4: Variazione del coefficiente di distribuzione K_D della vanillina e dell'acido ferulico in presenza di MeOH. La percentuale in volume del MeOH è riferita al volume della fase polare. Gli esperimenti sono stati condotti a 160°C per 20 minuti con Ar e 50 mM di POM-Mo.

ferenza si è potuta riscontrare nella selettività di reazione: solo in poche unità percentuali si è identificata la presenza del 4-idrossi-3-metossibenzoato di metile. Un altro aspetto interessante è che all'aumentare della concentrazione di MeOH si ha una diminuzione della produzione di vanillina come prodotto di reazione e la comparsa di acido ferulico come principale prodotto di reazione.

Altro aspetto degno di nota è l'andamento del coefficiente di ripartizione della vanillina e dell'acido ferulico all'aumentare della concentrazione di MeOH. Nella fig.1.4 si può osservare che all'aumentare della concentrazione di MeOH il K_D sia della vanillina che dell'acido ferulico diminuisce drasticamente, arrivando a valori minori dell'unità.

Al fine di variare il K_D dei prodotti di reazione, si è deciso di cercare di variare la loro solubilità inserendo un sale nell'ambiente di reazione: la scelta è ricaduta sull'NaCl il quale è economico,inerte ed con una discreta solubilità nella soluzione acqua-MeOH [19, 20]. Quindi, si è aggiunto al sistema con 40 vol.% di MeOH 250 mg di NaCl, quantità scelta al fine di rimanere al 70% dalla teorica saturazione della soluzione acqua-MeOH : il valore di solubilità utilizzato è pari a 2.665 mol/kg per una soluzione acquosa al 40 vol.% MeOH e il valore di densità utilizzato della soluzione è pari a 943.3 mg/ml [21]. I risultati hanno mostrato che la presenza del sale permette un amento della resa di reazione e una diminuzione della solubilità dei prodotti di reazione nella fase acquosa: in assenza di sale si era ottenuto una resa totale media monomerica pari a 3.67 wt.% e, invece, in presenza di NaCl si è ottenuto una resa di 4.48 wt.%, il che significa un aumento del 22 %. Inoltre i valori di K_D per la vanillina e per l'acido ferulico sono aumentati rispettivamente del 46% e del 69% rispetto agli esperimenti condotti in assenza di NaCl, ovvero ottenendo un K_D pari a 1.34 per la vanillina e 1.72 per l'acido ferulico. Si può concludere che diminuendo la solubilità dei prodotti di reazione nella fase polare, la presenza della fase organica permette effettivamente una migliore valorizzazione della lignina in composti aromatici.

1.5 Conclusioni

L'obbiettivo di questa tesi è nella ricerca di valevoli opzioni alle risorse petrolifere per la produzione di prodotti chimici. La lignina Kraft ricopre ben l'80% della produzione mondiale della lignina, e quindi risulta palese l'utilità nella potenziale scoperta per una tecnica che permetta una sua valorizzazione.

In questo progetto si è cercato di designare un sistema bifasico per la depolimerizzazione della lignina in composti aromatici in presenza di POMs. I risultati hanno mostrato come il sistema bifasico permetta di ottenere delle rese di reazione nettamente maggiori rispetto al corrispettivo sistema monofasico. Inoltre, il POM-Mo è risultato essere il più promettente, in termini di rese monomeriche, tra i POMs analizzati. Gli esperimenti condotti con la vanillina come materiale di partenza hanno dimostrato che la presenza del toluene come seconda fase in presenza di POM-Mo permetta una protezione della vanillina dalla sua degradazione/ripolimerizzazione durante il tempo di reazione.

L'effetto della presenza di MeOH sulla resa di reazione, selettività e solubilità dei prodotti di reazione è stata studiata. Si è osservato come la presenza di MeOH all'80 vol.% abbiamo portato ad una resa del 8.6 wt.% rispetto a 2.7 wt.% in sua assenza. La presenza del MeOH ha anche agito sulla selettività della reazione: all'aumentare della quantità di MeOH aggiunto si è avuto una diminuzione della quantità di vanillina prodotta e la comparsa dell'acido ferulico come nuovo e principale prodotto di reazione. Tuttavia, l'aspetto estremamente negativo dovuto alla presenza del MeOH è stato il drastico aumento della solubilità dei prodotti di reazione nella fase polare, arrivando a dei valori di K_D per la vanillina e per l'acido ferulico minori dell'unità. Per ovviare a tale problema, si è cercato di giocare sulla solubilità dei prodotti di reazione aggiungendo NaCl: l'aggiunta di questo sale nel sistema con il 40 vol.% di MeOH ha portato ad un incremento della resa del 22% e una diminuzione della solubilità dei prodotti di reazione nella fase polare.

Gli esperimenti condotti in questo studio hanno mostrato come il sistema bifasico sia una valida opzione da investigare per la depolimerizzazione della lignina. Tuttavia, i risultati ottenuti non permetterebbero uno scale-up industriale date le rese non abbastanza elevate. Inoltre, varie domande scientifiche rimangono aperte come. ad esempio, la variazione della selettività di reazione e la produzione di acido ferulico in presenza di MeOH. Rimane estremamente interessante la possibilità di studiare l'effetto della presenza di altri sali e quali risultati fornirebbero altre lignine tecniche.

2 Introduction

Nowadays, the climate problem and the depletion of fossil resources are increasingly leading the challenge of seeking renewable resources for the replacement of the current oil and gas economy. The treatment of biomass for this purpose is a scientific topic that has been the subject of several studies. However, energy production has a considerable options in the field of renewable energy, ranging from solar energy to wind power. A significant problem is, instead, the replacement of refineries and consequently the production of fine chemicals that are usually obtained from oil. In this context this thesis is included, i.e. in the search for a method for the valorisation of lignin in fine chemicals, and in particular in aromatic compounds.
The aim of this section is to provide a brief overview of what lignin is. Its chemical composition and extraction methods used in industry will be discussed. Finally, a presentation of the various techniques used in the literature for lignin depolymerization into aromatics is proposed.

2.1 Lignocellulosic biomass

Lignocellulose has been identified as the most abundant biomass on earth and therefore an inexhaustible and renewable resource for the production of fossil fuels and fine chemicals [1, 2]. It is the major component of plants, both herbaceous and woody, and its composition varies depending on the species, age of the plant and the growing conditions. The lignocellulose is predominantly composed by three different biopolymers which have the following fraction by dry weight: cellulose (40-60%), hemicellulose (10-40%), lignin (15-30%)[1, 3, 4]. In Tab.2.1, as an example, different percentages of the three biopolymers related to the nature of lignocellulosic biomass are shown. Cellulose and hemicellulose are polysaccharides [1], whereas lignin is the main natural polymer with aromatic backbone [22].The schematic arrangement of the three biopolymers inside the plant cell wall is proposed in Fig.2.1. The carbohydrate portion of lignocellulose is called holocellulose, which includes not only cellulose and hemicellulose but also components present in a minor fraction such as pectins.

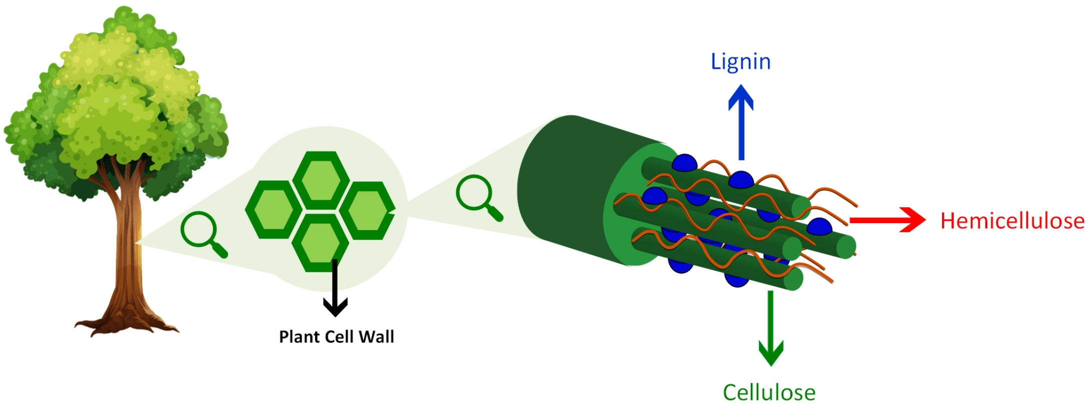


Figure 2.1: Schematic representation of the composition of the lignocellulosic biomass.

Lignocellulosic materials	**Cellulose %**	**Hemicellulose %**	**Lignin %**
Hardwoods stems	40-50	24-40	18-25
Softwood stems	45-50	25-35	25-35
Nut shells	25-30	25-30	30-40
Leaves	80-95	5-20	0
Corn cobs	45	35	15
Grasses	25-40	35-50	10-30
Waste papers from chemicals pulps	60-70	10-20	5-10
Paper	85-99	0	0-15
Newspaper	40-55	25-40	18-30

Table 2.1: This table gives an overview of the different compositions of lignocellulosic biomasses. The data have been taken from [4].

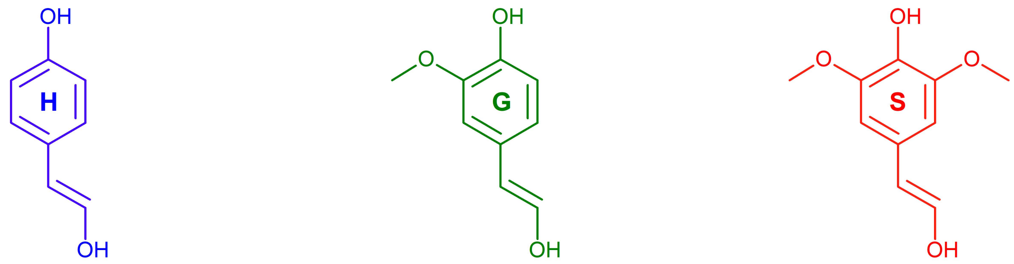
2.1.1 Cellulose and Hemicellulose

Cellulose is a biopolymer with a high degree of polymerisation (more than 10 000 units [23]) and its particular characteristic is to be composed only of D-glucose units linked together by a β-1,4 glycosidic bond, giving rise to long linear chains [1]. It is a semi-crystalline polymer due to the prensence of strong inter-molecular bonds (both hydrogen bonds and van der Waals forces). These secondary linkages allow the linear cellulose chain to be ordered in fibres, which provide rigidity to the plant). The cellulose is characterized by a insolubility in water and non polar organic solvents and it seems to be mainly due to the strong intermolecular bonds [24].

The second major component of lignocellulose is hemicellulose, which is a heterogeneous and branched polysaccharide. It contains both pentoses and hexoses sugars [22]. Its degree of polymerization is lower than the one of cellulose and it is between 50-300 units [1].
The third most important component of lignocellulose biomass is lignin. In contrast the others two major biopolymers, lignin is an highly heterogeneous and irregular biopolymer and it consists of methoxylated phenylpropanoid subnits [1].

2.1.2 Lignin

Lignin is an amorphous biopolymer and it is characterized by an high heterogeneity and irregular structure [1]. It can be found in the plant cell wall and it acts as a glue between cellulose and hemicellulose: it guarantees rigidity and strength to the cell wall and protect from microbial attacks [22, 8]. Its structure is extremely complicated and its fundamental units, called monolignols, can be identified in: p-coumaryl alcohol (4-hydroxycinnamyl), coniferyl alcohol (3-methoxy 4-hydroxycinnamyl), and sinapyl alcohol (3,5-dimethoxy 4-hydroxycinnamyl). As can be seen from the Fig.2.2, the three monolignols differ in the numbers of methoxy groups attached to the aromatic units [1, 25]. In the cytosol, the phenylpropanoid pathway allows the beginning of the synthesis of the monolignols, which



(a) **(b)** **(c)**

Figure 2.2: Raperesentation of the monolignonls, (a) p-hydroxyphenyl, (b) guaiacyl, (c) syringil which are respectvely abbreviated with H, G, S.

are then transferred to the cell wall where the lignification takes place. Lignification is initiated by the formation of radical phenolic units formed by the oxidation of the OH-groups by the enzymes laccase and peroxidase [1]. Then liginification occurs through polymerization reactions giving rise to the structure of lignin charterized by an high molecular weight.

The monolignols in the polymer structure are referred as p-hydroxyphenyl (H), guaiacyl (4-hydroxy-3-methoxyphenyl) (G), and syringyl (4-hydroxy-3,5-dimethoxyphenyl) (S).

The percentage of the various monolignols varies according to the plant species, thus giving rise to different lignin structures: usually in softwood lignin is composed of G units, while hardwood lignin is composed of both G and S, and finally the herbaceous biomass contains all three units but with low H percentages [1, 4, 22].

In lignin there are different types of bonds: first of all, the best known and usually the most abundant is β-O-4 from the family of ether bonds whilst another significant percentage presents carbon-carbon bonds [1, 5]. The percentage of the different types of linkages is closely related to the plant species, and therefore to the proportion of H/G/S units present in the plant structure [1].

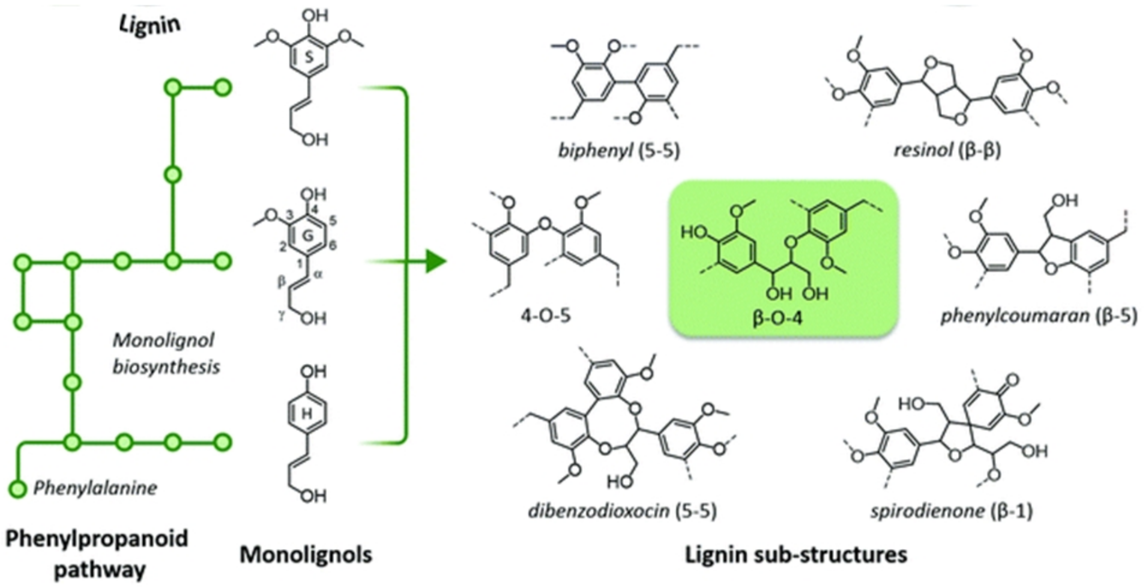


Figure 2.3: Representation of the main linkages present in lignin. Figure taken [1].

Linkage	Softwood Lignin	Hardwood Lignin
β-O-4	49-51	65
α-O-4	6-8	-
β-5	9-15	6
β-1	2	15
5-5	9.5	2.3
4-O-5	3.5	1.5
β-β	2	5.5

Table 2.2: Types and frequencies of linkages in Softwood and Hardwood Lignins (number of linkages per 100 C9 Units) [5].

2.2 Lignocelulose Fractionation

The fractionation of lignocellulosic biomass is a step of fundamental importance to take into account for a proper valorization of lignin, due to the complicated structure of lignocellulosic biomass. The type of fractionation used inevitably influences the chemical properties of lignin and consequently its reactivity in depolymerization. For this reason, lignin extracted from biomass is called technical lignin, i.e. commercially available lignin. In this section, the main fractionation methods used at industrial level will be analysed, with particular emphasis on the resulting properties of the extracted lignin.

Fractionation methods can be divided into two main classes: processes with the main purpose of delignifying lignocellulosic biomass, i.e. releasing the portion of cellulose and hemicellulose from lignin, and processes that directly convert and solubilize biomass carbohydrates.

2.2.1 Kraft Process

The Kraft process is the largest technique used for the pulping process and provides 80% of the world's pulp production, which means that 50-55 tonnes of Kraft lignin are produced every year [6].

In Fig.[26] is proposed a slow-sheet of the Kraft process. The lignocellulosic biomass is fed to the process as wood chips and it undergoes digestion for a few hours at a moderate temperature (145-170°C) in presence of sodium hydroxide (NaOH) and sodium sulfide (Na_2S) [1, 7]. The resulting solution is called white liquor [1, 7]. The extremely high pH favours biomass delignification and lignin solubilization, breaking the carbohydrate-lignin bonds. At the same time, the extreme alkaline environment causes the rupture of the β-O-4 bonds of lignin, leading to solubilization of the fragments and a severe degradation and repolymerization [1, 7]. In this step of the process, about 90% of the lignin is removed from the biomass. Then the carbohydrate fraction is separated and the resulting solution where lignin is dissolved is called black liquor. Usually black liquor is incinerated in order to produce steam as a hot utility for the process and inorganic compounds such as $CaCO_3$ and CaCO are recovered from the bottom of the furnace [6]. Alternatively, lignin can be isolated from black liquor and this process is usually carried out by precipitation through acidification of the solution [1, 8, 6]. One of the most famous processes of isolating lignin

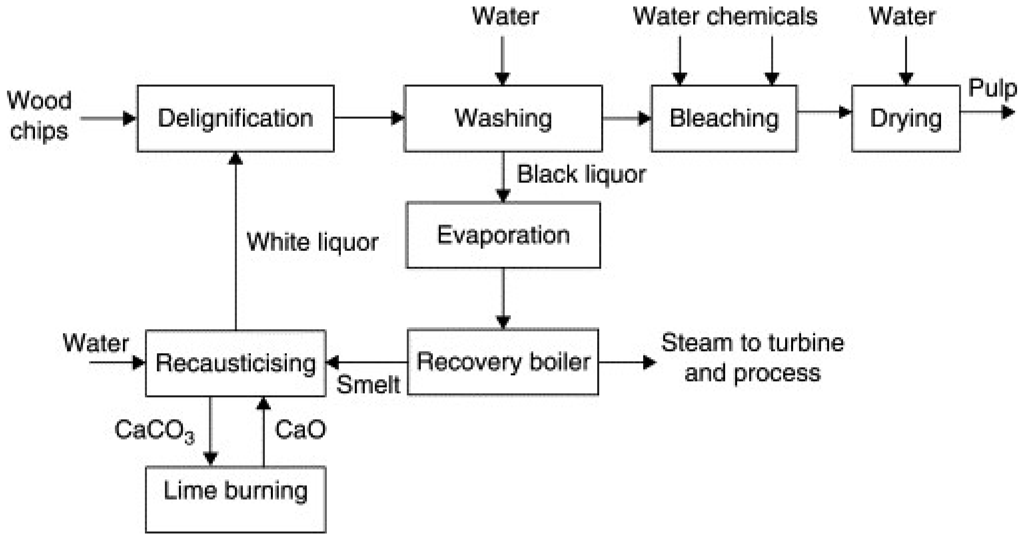


Figure 2.4: Simplified Kraft process flow-sheet [26].

from black liquor is LignoBoost: this process involves multi-steps acidification with CO_2 and H_2SO_4 [6].

2.2.2 Soda Process

Soda pulping is a process that dates back to 1851 and consist of the treatment of lignocellulosic feedstock with a NaOH aqueous solution in a batch reactor at 160-170°C [27, 28]. These operating conditions lead to the degradation not only of lignin but also of cellulose and hemicellulose [1, 27]. For this reason, this process is usually used for the treatment of biomasses with low lignin content, such as herbaceous feedstocks. The process has been improved with the addition of anthraquinone, which enables a reduction in the degradation of the carbohydrate fraction [28]. The lignin can be recovered simply by precipitation acidifying the black liquor, and the main feature is the absence of sulfur but nevertheless the soda lignin is extremely condensed, with low ether linkages [1, 28].

2.2.3 Sulfite Process

Sulfite pulping is the second most common method of lignocellulose fractionation, but it is a very small part of the world market ($\leq 5\%$) [1]. In 1874, this technique was used for the first time in Sweden, but today this process has been almost completely replaced by the Kraft process which has been recognized as more efficient and versatile due to the ability to easily adapt to the treatment of different types of wood [1, 6]. The sulfite process involves the use of sulfite/bisulfite salts, which independently of the pH (the pH can vary from 3 to 13) allow the solubilization of lignin forming compounds called lignosulfonates [4, 7]. The salts usually used are acid sulphates($HSO_3{}^-$) or sulphates ($SO_3{}^{2-}$), but also sulphur dioxide (SO_2) and sulphuric acid (H_2SO_4) can be used. The cation of the salt can be an alkali metal (Na^+ and K^+), alkali earth metal (Mg^{2+} or Ca^{2+}) or ammonium ion (NH^{4+}) [6]. It is extremely remarkable that the presence of these salts make the lignin fragments soluble in the medium independently of the pH, so it is obvious that the isolation of the lignin fraction cannot occur by simple precipitation via acidification [1, 6].

2.2.4 Organosolv Process

The Organosolv process is a process, as its name suggests, involving the use of organic solvents (pure or in aqueous solution) to perform the fractionation of lignocellulosic feedstock. The main problem of this process lies in the price of these organic solvents [6, 2]. The organic solvents used can be of different types, such as alcohols, cyclic ether, organic acid. In order to reduce the costs of this process, the solvent is usually recovered by evaporation, therefore a suitable solvent for this process has to be at the same time low cost and volatile, and ethanol shows ideal characteristics [7]. The Organosolv process can be performed over a wide temperature range (100-250 °C) and usually at high pressures [6]. The organic solvent allows the solubilization of the biomass and in particular the isolation of the three main polymers takes place without aggressively affecting the structure of the lignin. The process provides cellulose in the form of solid pulp, lignin as precipitate, and an aqueous solution in which hemicellulose is located [1, 6]. It is important to emphasize that this process is sulfur free, and therefore even the extracted lignin will be free of it allowing a better exploitation of this polymer given the poisonous nature of sulfur for catalysts [6]. It has been discovered that the Organosolv process in the presence of high concentrations of alcohols allows a lignin extraction with better preservation of the β-O-4 bonds: the alkyl groups deriving from the solvent bind in alpha position of the lignin allowing a stabilization of the bond, as shown in Fig. 2.5 [1, 29].

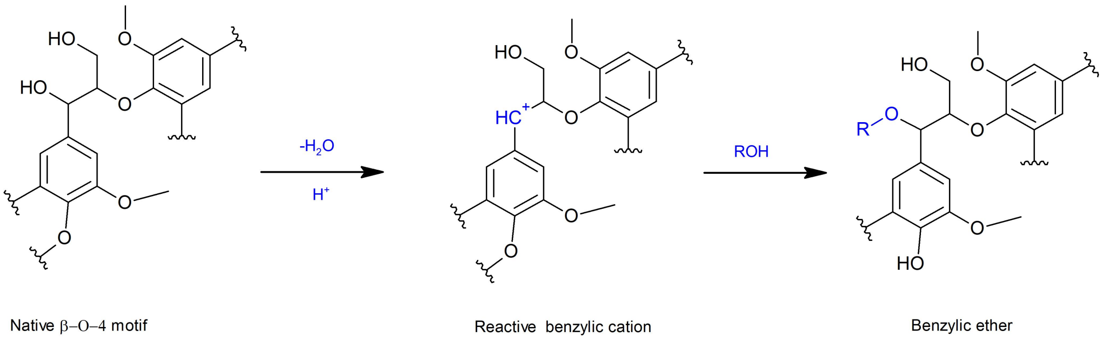


Figure 2.5: Alkylation in α-position of lignin's alkyl chains in presence of concentrated alcohols [1].

2.2.5 Lignin Isolation for a future biorefinery

In the past, processes for lignocellulosic biomass fractionation have been developed with the main purpose of exploiting the carbohydrates and not paying particular attention to the lignin macromolecules. In recent years this biopolymer has received particular interest from the scientific world and a research group at *École polytechnique fédérale de Lausanne* (EPFL) claims that to integrate it in an economically sustainable way and to fully exploit the lignin value it is necessary to carry out the fractionation method which is able to preserve the identity of all three biopolymers. The result of their research was a new lignin extraction method that involves heating the biomass to mild temperatures (75-100°C) in a 1-4-dioxanes solution in the presence of HCl (0.3-1.4M) [30, 31]. The key element of this method is the addition of aldehydes (formaldehyde or propionaldehyde), which prevent the formation of C-C bonds. Aldehyde allows the formation of a stable structure, reacting with the two free diols forming the cyclic unit 1-3-dioxane [30, 31]. In addition, methylation reactions of the benzene rings also occur [30, 31]. A reaction scheme is proposed in Fig. 2.6. In order to test the extraction method, the lignin extracted in the presence of aldehyde was subjected to subsequent hydrogenolysis at temperature of 250°C for 15h resulting in a monomeric yield close to the theoretical ones [30, 31].

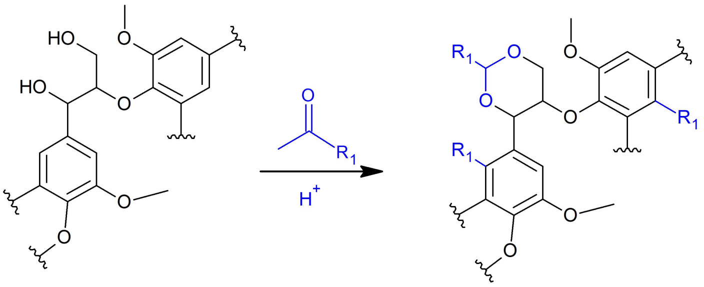


Figure 2.6: Proposed mechanism of lignin stabilization during the fractionation in presence of formaldehyde [1].

2.3 Biorefinery

Nowadays, the urgent necessity to become independent from fossil resources is becoming more and more a priority. Oil resources not only provide energy, but about 5% of oil and gas production goes to petrochemicals [32]. Therefore the need of the development of biorefinery is becoming imperative, which can be defined using a definition provided by IEA Bioenergy Task 42: "Biorefining is the sustainable processing of biomass into a spectrum of marketable products (food, feed, chemicals, and materials) and energy (biofuels, power and/or heat)" [33]. With this in mind, a biorefinery should be designed to replace fossil resources with biomass able to be converted into fuel and fine chemicals. A biorefinery can be categorized as 1st or 2nd generation, depending on the type of biomass is treated: 2nd generation biorefineries, in contrast to 1st generation ones, deal with biomass that does not compete with the food sector [34]. A suitable and abundant biomass that does not compete with food is lignocellulosic one: processes for the valorization of cellulose and hemicellulose are already possible, as they can be hydrolyze into sugars and consequent fermentation in ethanol. In a concept of renewable energy, the ideal would also be to be able to carry out a valorization of lignin, such as its conversion into aromatic substances, instead of its simple combustion, which is the most used procedure in industry today.

2.4 Overview of lignin depolymerization into aromatic compound

The depolymerization of lignin, and therefore its enhancement is a topic that has received considerable interest and attention in order to find the best way to integrate it into a concept of biorefinery. As discussed in the previous sections, the chemistry of lignin is extremely complicated as it is influenced primarily by its botanical origin, but also by the method of extraction applied. Furthermore, depolymerization can be performed in the presence of a homogeneous or heterogeneous catalyst and pre-treatments of lignin can be applied, for example in order to increase its solubility. Therefore, it is clear that it is a difficult task to provide a complete overview of the different methods of depolymerization since a multitude of parameters can be varied.

A generally accepted classification divides lignin depolymerization methods into five categories [1, 35]: (i) acid/based -catalyzed, (ii) oxidative, (iii) reductive, (iv) solvolytic and (v) thermal depolymerization.

2.4.1 Acid/Based-catalysed depolymerization

Acids have historically been used in the pulp industry as they allow the hydrolysis of the ether bonds of the carbohydrates cellulose and hemicellulose, but lignin is insoluble and indeed the acid environment leads to a severe modification of its structure [36]. In an acidic environment, the ether bonds of lignin are cleaved resulting in a carbocationic benzyl intermediate [1, 37, 38]. This intermediate can give rise to enol ethers, or it can proceed in repolymerization pathaway [1, 37, 38]. The enol ethers can therefore provide monomers or fall within the depolymerization pathway [1, 37, 38]. Others studies show that the kind of acid applied can influence a privileged pathway or another [37, 38].

The acid-catalyzed depolymerization of lignin has been extensively studied by testing soluble and insoluble Lewis and Bronsted acids in aqueous solution [1]. As lignin is insoluble in acidic environments, several researches have been carried out in pure organic solvents or in a mixture with water, such as methanol(MeOH), ethanol, butanol, and dioxanes, resulting in greater solubility of the biopolymer and higher reaction yields [1]. Usually the temperatures applied are quite high (≥250°C) [1], however studies with model compounds have demonstrated that such high temperatures are not necessary to perform the rupture of the ether bond β-O-4, as this occurs already at 85°C [37, 39].

Base-catalysed depolymerization of lignin, as for acid-catalyzes, has been studied in presence of soluble (i.e. NaOH, MgOH) and insoluble bases (i.e.MgO, CaO) using as solvent either pure water or mixtures of organic solvents [27, 36]. The operating temperatures are generally quite high (240-330°C) and the temperature applied has an influence on the selectivity of the reaction products: for temperatures below 300°C are usually produced mainly methoxyphenols, while at higher temperatures catechols (or alkylcathecols) [1].

2.4.2 Oxidative depolymerization

Oxidative lignin depolymerization involves the presence of an oxidized agent, which is usually oxygen or hydrogen peroxide. The reaction products can be either phenolic compound or aliphatic caboxylic acid. This process can be conducted in both basic and acidic environments. To date the only process that has been able to perform an industrial scale-up: from the 50s to the 70s the production of vanillin from lignosulfonate was the predominant technique in the market. However, today the production of lignosulfonate has been almost completely replaced by the kraft process, and the only company still producing vanillin from lignosulfonate is Borregaard Lignotech, which accounts in 2004 for 12,5 % [40] of world vanillin production. Oxidation in a basic environment is usually carried out in the presence of NaOH, or more rarely KOH, usually at a concentration of 4M [1]. Reaction temperatures are not very high and usually vary between 120°C-190°C and with a partial oxygen pressure between 2-14 bar. Reaction yields are usually between 10-20%. The system has also been studied in the presence of soluble (e.g. $CuSO_3$, $FeCl_3$,$CoCl_2$) and insoluble (CuO, Fe_2,Pt/Al_2O_3, Pd/Al_2O_3) catalysts. Lignin oxidation can also be conducted in an acidic environment. Catalysts used are both soluble and insoluble, generally providing lower yields than the corresponding process in an alkaline environment[1].

2.4.3 Reductive depolymerization

The reductive depolymerization of lignin is based on the use of redox catalysts and a reducing agent. The type of reducing agent applied allows a first classification of this methodology: if pure hydrogen is present, the process is called hydroprocessing, if instead hydrogen enters the reactive environment for solvent donation it is called liquid-phase reforming [1, 35].

During mild hydroprocessing, lignin is dispersed in a liquid phase (which can be water, organic solvents or a mixture of the two) with a pressure of H_2 between 10-100 bar [1, 41]. The reaction temperature is under 300°C and this mild temperature allows the preservation of methoxyl groups obtaining methoxyphenols as main reaction products. The redox catalysts used are both noble metals and metal base. The presence of co-catalysts such as Lewis acids or bases has shown an increase in monomer yields. Instead, hard hydroprocessing is generally conducted at temperatures between 300°C and 450°C, with H_2 pressures between 35-100 bar in the absence of solvent [1, 41]. Catalysts are noble metals or metal based. These high temperatures do not allow the preservation of methoxy groups and therefore the products of reactions are phenol and methylated phenols [1]. Liquid phase reforming is conducted in the presence of hydrogen-donating solvents such as formic acid, tetralin and organic alcohols (MeOH and ethanol). The catalysts used are the same as for hydroprocessing. A crucial point to be investigated in this technique is the regeneration of the solvent in a perspective of sustainability of the process.

2.4.4 Solvolytic depolymerization

The process called solvolytic depolymerization involves a lignin treatment in absence of a catalyst: the depolymerization takes place through the combined effect of the action of the solvent and the medium-high temperatures (250-450°C) [1, 36, 42]. The solvents used may be pure water, organic solvents or a mixture of the two. The data generally available for this technique comes from blank experiments carried out in order to investigate catalytic activity of the system. In general, it has been shown that better reaction yields are obtained in the presence of the corresponding systems in the presence of catalysts [42].

2.4.5 Thermal depolymerization

Two types of depolymerization are included in the thermal treatments of lignin: fast pyrolysis and catalytic fast pyrolysis. Both techniques involve heating lignin to extremely high temperatures (400-800°C) without solvents and O_2 [1, 36]. During this process a solid rich in carbon and vapours are obtained; the vapours are then subjected to condensation at the outlet of the reactor giving rise to an oil. Fast pyrolysis differs from catalytic fast pyrolysis due to the absence of a catalyst and therefore the data in the literature represent the blank runs of catalytic fast pyrolysis. The catalysts most frequently used in catalytic fast pyrolysis are acid zeolites and mesoporous silicas [1, 36, 42, 43]. The catalysts can be placed either in the reactor together in the biomass or they can be in contact only with vapours. The presence of catalysts has been shown to stabilize reaction products and to ensure greater selectivity of reaction products by mainly obtaining deoxygenated aromatic products such as benzene, xylene, toluene [42, 43]. One of the main problems of this technique lies in the scale-up of the process: lignin has a low melting point and tendency to agglomerate and therefore there are problems of plugging in the fluidized bed reactor [1].

2.5 Aim of the research

The aim concerning this thesis is the designation of a biphasic system for the depolymerization of Kraft lignin in aromatic compounds at mild temperatures. This work is part of the "Green chemicals from lignin" project of the Catalytic Process Engineering Group, Paul Scherrer Institut, Switzerland. The idea is to design a system that allows the depolymerization reaction to take place at the interface between the polar and apolar phases, allowing the reaction products to be extracted in the apolar phase and therefore protected from condensation and repolymerization reactions, which are quite frequent during lignin depolymerization. An article published by this research group has already shown the potential of a biphasic system compared to a simple aqueous system: they tested a water-octanol system in the presence of H_2SO_4 which resulted in a vanillin yield six times higher than the corresponding experiment conducted in an aqueous medium [9]. The experiments within this thesis are related to the design of a new biphasic system in the presence of polioxometalates (POMs) as catalysts, which act as stoichiometric oxidizers in the absence of O_2. In particular, different solvents, two different POMs and the reaction temperature will be analyzed, highlighting critically and comparing the results with a system in the presence of H_2SO_4 and other researches presented in literature. Moreover, the role of MeOH as radical scavenger and the solubility of monomeric products from lignin depolymerization will be investigate in order to obtain higher yields, as will be outlined in chapter 4.

3 Tools & Setup

In this section, the experimental methods used during the thesis will be presented. First of all, a list of the substances and the type of lignin used are introduced, underlining the biomass properties. The set-up used for the preparation of the experiments is described in conjunction with the procedures with which the experiments are prepared and performed. Finally, the analytical procedures, identification of target reaction compound, their quantification and the methodology used to carry the data analysis are explained in detail.

3.1 Chemicals

A comprehensive list of the chemicals used during this thesis is provided in the Tab.3.1. Milli-Q water produced in the lab was used during the experiments. Laboratory observations showed that Phosphomolybdic acid (POM-Mo) and Tungstosilicic acid (POM-W) have a negligible solubility in toluene, and these compounds were used as catalysts. For this reason, in this research the catalyst concentrations will always refer to the volume of the polar phase.

Compound	**CAS Number**	**Sum Formula**	**Supplier**
Acetonitrile	75-05-8	C_2H_3N	Merk
Methanol	67-56-1	CH_3OH	Fisher Scietific AG
1-Octanol	111-87-5	$C_8H_{18}O$	Sigma Aldrich
Phosphomolybdic acid	51429-74-4	$H_3PMo_{12}O_{40}$	Sigma Aldrich
Sodium chloride	76-47-14-5	NaCl	Sigma Aldrich
Sulphuric Acid (98 wt.%)	7664-93-9	H_2SO_4	Supelco
Syringaldehyde	134-96-3	$9\,H_{10}O_4$	Sigma Aldrich
Tert-Butanol	75-65-0	$C_4H_{10}O$	Sigma Aldrich
Toluene	108-88-3	C_7H_8	Merk
Tungstosilicic acid	12027-43-9	$H_4Si_4W_{12}O_{40}$	Sigma Aldrich
Vanillin	121-33-5	$C_8H_8O_3$	Sigma Aldrich
Vanillin-(phenyl-13C6)	201595-58-6	$^{13}C_6H_8{}^{12}C_2O_3$	Sigma Aldrich

Table 3.1: A comprehensive list of the chemical used for the experimental and analytical work.

3.2 Lignin Source

The aim of this research is the conversion of lignin into high value added chemicals, therefore it has been decided to use one of the most commercial lignin: softwood Kraft lignin Indulin AT (softwood) was provided by MeadWestvaco, whose water content has been considered to be 3.5%wt..

As already discussed in the section 2, the chemical and physical properties of lignin are strongly influenced by the method used for its extraction. Softwood Kraft lignin is a biopolymer with a high average molar mass ($M_W = 4290$) and it is highly polydisperse (polydispersity index equal to 8.1) [10]. Softwood Kraft lignin is soluble in alkaline environments, like aqueous solution of NaOH, instead it is almost insoluble in acidic environment even if it has been reported that at high temperatures lignin become more soluble in acidic solution and also in pure water [1, 10]. One of the main parameters used to characterize lignin is the percentage of monolignols, S,G,H present in the biomass. A study reported that softwood Kraft lignin is composed almost exclusively of G units: out of 100 aromatic units of lignin 97 are G, 3 H and S unit has not been identified [10]. In addition, softwood Kraft lignin is characterized by the presence of 7.1 ether bonds out of 100 aromatic units, of which 6.1 are β-O4 bonds [10]. The β-O-4 bond usually is the target bond of lignin depolymerization, as C-C bonds are more difficult to break: note that it has been reported that native softwood lignin is characterized by a 45% β-O-4 bond out of 100 aromatic units [44]. Therefore, the lignocellulosic fractionation process has a significant impact on the resulting lignin structure and consequently on its potential enhancement. One of the major problems related to the treatment of Kraft lignin is the presence of sulfur, which is well known to be poisonous for catalysts: in the literature it is reported that the sulphur content is about 2 wt.% of the biomass. [10].

3.3 Parameters screening

In this study, the reaction volume, lignin concentration and reaction time were kept constant. The type of catalyst, its concentration, temperature and solvents were varied. The reaction volume was always 5 ml, of which:

- 2.5 ml of an apolar phase and 2.5 ml of a polar phase in case of experiments conducted in a biphasic system;
- 5 ml of polar phase in case of experiments conducted in a monophasic system.

The concentration of lignin used is 1 mg/ml, which mean 5mg of lignin. The system is then heated to the desired temperature for 20 minutes. The experiments were all conducted in triplication.
In order to have a structured work, an experiment labelling system based on ShortName_number-of-experiment_A/B/C was used: the "ShortName" is simply for the identification to who belongs the sample in the lab, the number was assigned sequentially according to the order in which the experiments were carried out, whereas the letters indicate that it is one of the triplications of the same experiment.

3.4 Microwave Batch Reactor

The microwave batch reactor used in this research is the Biotage® Initiator+. The reaction take place into a glass vial with a volume of 10ml which can be filled to a a maximum of 5ml. It is possible to imagine the development of experiments with the microwave batch reactor divided into three phases:

1. Preparation of the vial: the catalytic environment desired to be observed is inserted into the vial;

2. Reaction: The vial is inserted into the microwave and heated up for the desired;
3. Recovery of the phases: open the vials with a de-crimper and recover both phases.

The first step is described in Appendix A, where a detailed description of the procedure used for each kind of experiments is listed. When the vials is closed, it is necessary to substitute the air present into the vials with the desired gas.

Time
00:05:00

Temperature
300°C

Pressure
Off

Pre-Stirring
00:00:00

Vial Type
2.0-5.0 ml

Absorption Level
Normal

Fixed Hold Time
On

Temperature
300

(a)

(b)

(c)

(d)

Figure 3.1: Microwave batch reactor: (a) is representative of the microwave Biotage® Initiator+, (b) is the GUI of the microwave through which it is possible to set up the experiment with the desired parameters, (c) is the glass vial in which the reaction takes place, (d) represents where the vials is inserted in the microwave. The pictures have been taken from [45].

Therefore, two needles are inserted through the closed cap: one is used to influx gas into the vial and the second one is used to avoid pressure buildup (this is a needle inserted into the vial connected to the lab environment). The gas is fluxed through the system at room temperature and pressure over a period of ten minutes, after which it is assumed that saturation is achieved. The needles are removed and the vial is placed inside the microwave.

The reactor is equipped with a simple GUI that allows the user to choose the reaction temperature and reaction time. It is characterized by an heating rate of 2-5°C/s and the vials are magnetically stirred at 600rpm. It is also possible to monitor the pressure, temperature and power profile over the reaction time: the microwave is equipped with an IR sensor for temperature and the pressure is directly measured on the top of the vial. At the end of the reaction time, the vial is cooled with pressurized air. In Fig.3.1 is shown the microwave batch reactor, the graphic interface through which is possible to set the desired experiment condition and the glass vial where the reaction take place.

The vial is then opened and the two phases are recovered, filtered with a syringe equipped with a 22μm filter and stored in eppendorf and centrifuged for 10 minutes at 17 G.

3.5 Analytical Methodology

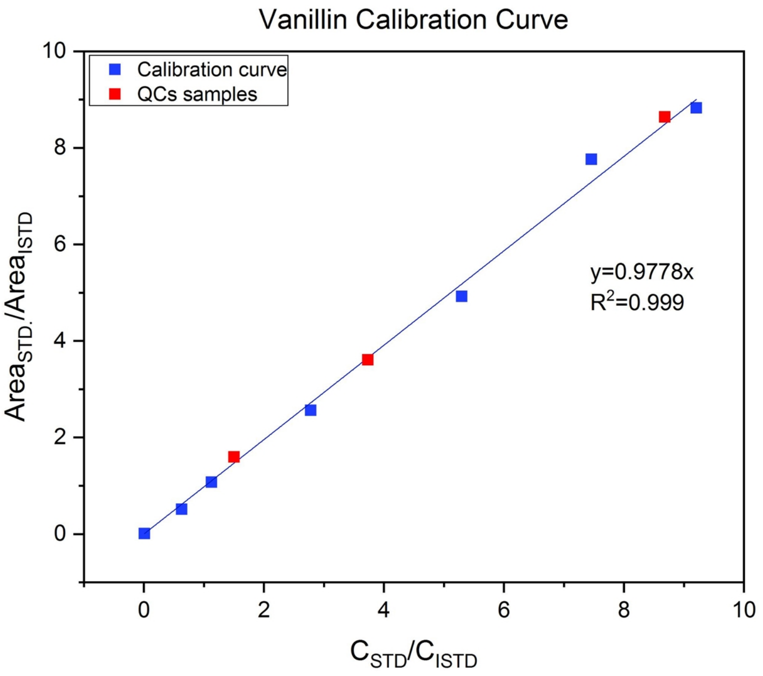
The analytical instrumentation used to perform the identification of the reaction products and its calibration will be described in this section.

3.5.1 UHPLC-HRMS

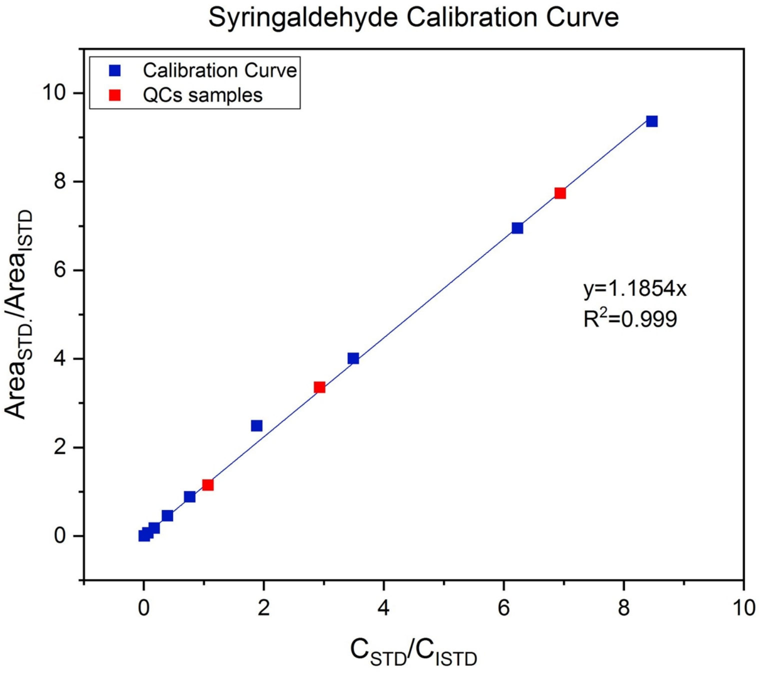
Identification and quantification of the monomeric product reaction has been performed using a UHPLC-HRMS. The separation of the substances took place in a Thermo Scientific Dionex Ultimate 3000 Series RS system (Thermo Fisher Scientific, Switzerland) and Separation of the analytes was achieved with a column ACQUITY UPLC C-18 VanGuardTM pre-column and column (150 mm x 2.1 mm x 5 mm, particle size 1.7 µm) from Waters (Switzerland). Data acquisition was performed using a Thermo Scientific Q-Exactive^{TM} hybrid quadrupole-orbitrap mass spectrometer controlled by Xcalibur 4.2 software (Thermo Fisher Scientific, Switzerland). The samples were run in positive mode, with electrospray ionization (ESI) as ionization source.

3.5.2 Calibration curve and quantification

The main part of the research is based on the quantification of the monomeric compound produced from lignin depolymerization. For this reason, particular attention was given in the preparation of the calibration curve. Two calibration curves were prepared, one for vanillin and the other one for syringaldehyde, which are shown in Fig. 3.2. Both calibration curves were prepared using ^{13}C-vanillin as internal standard. The choice of an internal standard may fall into either an isotope of the target substance or a structurally similar molecule. In our case, vanillin has been quantified with its isotope and the structural similarity of the isotope has been used to quantify the syringaldehyde. In addition, the quantification of the other substances identified was carried out assuming that they have the same response as vanillin. It has been found that the presence of an internal standard helps to avoid the matrix effect phenomenon: the signal of the substance and the one of the internal standard will be increased or suppressed in the same way, thus avoiding possible errors in quantification [46, 47].



(a)



(b)

Figure 3.2: Calibration curve for quantification through UHPLC-HRMS: (a) and (b) are the calibration curves for vanillin and syringaldehyde respectively. The x-axis is the ratio of the concentration of the calibrated substance (in this case vanillin or syringaldehyde) to that of the internal standard and the y-axis is the ratio of the areas of the calibrated substance to that of the internal standard. In blue are the points constructed for the calibration line, while in red are the quality control samples. The calibration curves were performed in a concentration range between 0.1μM and 100μM.

The calibration curves used in this study were built on the basis of nine different con-

centrations of the desired compound (100/75/50/25/10/5/2/1/0.1 μM), using an internal standard concentration of 10uM. During the preparation of the curves, each dilution was made using micropipettes and weighting the added amounts and note them down. In this way it was possible to calculate the exact concentration of the prepared solutions. Moreover, three quality control samples (QCs) were prepared in order to verify the quality of the curve: each QCs has been made from different stock solutions and the chosen concentrations are 85/35/15 uM.
The calibration curve obtained for vanillin has as the following equation:

$$y = 0.9778x \tag{3.1}$$

where y is the ratio between the area of vanillin and the area of the internal standard detected and x is the ratio between the concentration of vanillin and the concentration of internal standard.
The calibration curve obtained for syringaldehyde has as the following equation:

$$y = 1.1854x \tag{3.2}$$

where y is the ratio between the area of syringaldehyde and the area of the internal standard detected and x is the ratio between the concentration of syringaldehyde and the concentration of internal standard.
Both curves are characterized by an R^2 of 0.999.

3.5.3 Sample preparation for UHPLC-HRMS

No minor importance is given to the preparation of the samples to be analysed with LC-MS. The vials used for the analysis have a volume of 300 μL. All samples analysed have ^13C- vanillin as internal standard, which has been added from stocks solution of 11 μM in MeOH. The procedure used to prepare these samples can be summarized as follow:

- pipette 10uL of the phase to be analysed into a 300uL vial, and weigh this quantity;
- pipette 90 uL of the 11uM stock solution of ^13C- vanillin in MeOH and weigh the total amount present in the vial;
- close the vial with the appropriate cap;
- mix it for a few seconds with the help of the vortex mixer;
- visually check that there are no bubbles of air in the vial.

The quantities of both the solution to be analysed and the solution with internal standard were recorded in order to calculate the exact dilution coefficient and the concentration of ^{13}C- vanillin in the vial analyzed. On the basis of these two parameters the quantification is carried out, and for this reason it is necessary to be as accurate as possible.

3.6 Data Analysis

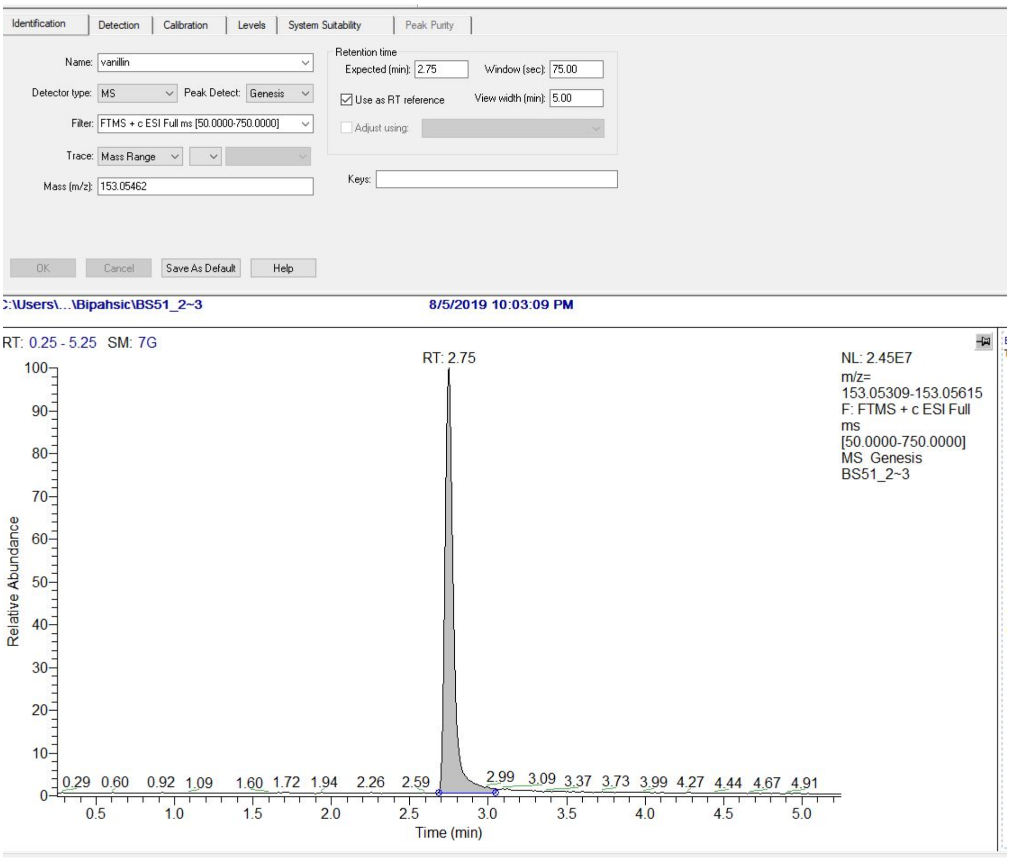
The raw data obtained from the analysis of the UHPLC-HMRS samples have been processed with two different software. First of all the raw data has been treated with Thermo Scientific^{TM} Compound Discover^{TM} 3.0: this software allows the identification of the substances present in the analyzed samples by comparison with two widely accepted libraries, i.e. mzCloud^{TM} and ChemSpider. Thanks to this software, it is possible to identify the main reaction products present in the analyzed samples. In this study ten different substances have been identified as the main reaction products. For some selected compounds it was only possible to identify the sum formula and not to associate a precise name to it: this is due to the fact that only a partial match was obtained with the two libraries named above and a total match in the prediction of the sum formula of the substance was achieved with the software. In order to identify with certainty the identity of such molecules it would be necessary to compare the spectrum of the various possible pure substances with the spectrum of the samples in which the unknown substance is present. In the table 3.2 is present a list of all the aromatic compounds selected to be quantified in the reaction mixture.

Sum Formula	**Compound**	**R.T.[min]**
$C_8H_8O_3$	Vanillin	2.85
$C_9H_{10}O_4$	Syringaldehyde	3.10
$C_9H_{10}O_3$	Apocynin	3.45
$C_9H_{10}O_4$	Methyl vanillate	3.63
$C_{10}H_{10}O_4$	Ferulic Acid	3.31
$C_8H_6O_3$	-	4.01
$C_7H_8O_2$	-	2.75
$C_7H_6O_2$	-	2.30
$C_{11}H_{14}O_5$	-	3.45
$C_{10}H_{10}O_5$	-	3.58

Table 3.2: List of the monomeric compounds detected during the lignin depolymerization reaction conducted in this study.

After the identification of the main substances, it is necessary to develop a method with the Thermo Fischer Xcalibur$^{4.2}$ software to process the raw data from the LC-MS by identifying the peak areas of all desired substances. In order to carry out an efficient method, the software allows to load a raw data from the LC-MS in order to have a preview if the selected settings identify the desired peak. Then enter in the "Name" section the name of the substance you want to identify, "Detector type" MS and "Peak Detect" Genesis. The selected filter is full MS [50.0000-750.0000] specific for the ESI ionization source. Then, mass to charge ratio is set to identify peak: it is important in this case to enter the mass of the compound desidered to be identified with an extra proton because all the results presented in this thesis have been run in positive mode. The time at which the substance is expected to elute is defined, giving a maximum window of time that is expected to vary (usually is set at 30 sec). In the detection window it is possible to set all the parameters about peak integration, such as sounds versus noise threshold and tailoring factor. In addition, in the "Calibration" section it is necessary to specify whether the substance to be integrated is a target compound or the internal standard.

Once the method is developed, it is possible to analyze the desired data: the software



Name: vanillin

Detector type: MS

Peak Detect: Genesis

Filter: FTMS + c ESI Full ms [50.0000-750.0000]

Trace: Mass Range

Mass (m/z): 153.05462

Retention time

Expected (min): 2.75

Window (sec): 75.00

☑ Use as RT reference

View width (min): 5.00

☐ Adjust using:

Keys:

:\Users\...\Bipahsic\BS51_2~3

8/5/2019 10:03:09 PM

RT: 0.25 - 5.25 SM: 7G

NL: 2.45E7
m/z=
153.05309-153.05615
F: FTMS + c ESI Full
ms
[50.0000-750.0000]
MS Genesis
BS51_2~3

Figure 3.3: Example of the interface of the software Thermo Fischer Xcalibur[4.2] during the method development.

allows to generate a list of raw data to analyze through the option "Sequence set-up" in which you specify the method with which you want to analyze the following results, which of course is the method previously developed. Then you start the raw data analysis by selecting the "Batch Reprocess" option. Then through the option "Quan broswer" you can manually check if the integration of peaks for each substance is done in the correct way, and if it is not, you can manually modify the integration or the integration settings. It is then possible to save a report in CSV format that is made of multiple sheets: each sheet represents the peak integration data as area, internal standard area, retention time, etc. for each raw data analyzed.

It is necessary to make calculations for data analysis. The calculations made and the formulas used are explained below:

- Calculate the exact dilution carried out in the preparation of the vial for LC-MS, i.e. the dilution factor D.F.:

$$D.F. = \frac{V_{exp}}{V_{Tot}} \tag{3.3}$$

  where the V_{exp} is the volume of one of the phases of the experiment inserted during the vial preparation, and V_{Tot} is the total volume in the vial calculated as the sum of V_{exp} and the volume of the stock solution ^13C-Vanillin in MeOH added to the vials ($V_{Stock-13C}$). Both these volumes were calculated from the masses noted during the preparation of the vials, as explained in section 3.5.3, and therefore the volume is calculated dividing the mass by the density of the solution. The densities used are those of pure solvents.

- the concentration of internal standard present ^13C-Vanillin (C_{ISTD}) in the vials analyzed, calculated as:

$$C_{ISTD} = \frac{V_{Stock-13C} C_{Stock-13C}}{V_{Tot}} \tag{3.4}$$

  where $V_{Stock-13C}$ is the volume, as previous explained, of the stock solution of ^13C-Vanillin and $C_{Stock-13C}$ is the concentration of the internal standard ^13C-Vanillin present in the stcok solution used for the preparationn of the vial.

It is possible to calculate the effective concentration of the target substances present in the samples analyzed by knowing the two parameters $D.F.$ and C_ISTD and having performed a calibration curve for the quantification of the targeted substances. In this studies two calibration curve have been developed one for vanillin and the other one for syringaldehyde, and the quantification of the other substances has been done on the hypothesis that their response is equal to the one of vanillin, and therefore has been used for their quantification the calibration curve of vanillin. Considering the calibration curve with the general formula $\frac{A_x}{A_{ISTD}} = a\frac{C_x}{C_{ISTD}}$ where A_x is the area of the target compound, A_{ISTD} is the area of the internal standard,C_x is the concentration for the target compound and C_{ISTD} is the concentration of the internal standard is possible to calculate the effective concentration of the target compound in the solution taking into account the dilution factor, as follow:

$$C_x = \frac{A_x/A_{ISTD}}{a}\frac{C_{ISTD}}{D.F.} \tag{3.5}$$

It is therefore possible to estimate the reaction yield normalized to the amount of lignin present in that experiment:

$$Yield_x = \frac{m_x}{m_{lig}0.965}100 \tag{3.6}$$

where $m_x$is the mass of the compound target compound x which is calculated considering the volume of solvent that was added in the microwave batch reactor, m_{lig} is the mass of lignin used in the experiment which is multiplied per 0.965 which takes into account a 25% of water presented in lignin.
In this research the experiments were performed in triplication, and therefore the data must be treated accordingly: for each final value presented, the mean value on the triplication of the experiments using the definition of arithmetic mean, the standard deviation and the coefficient of variation were calculated.

Other parameters have been calculated in order to have a first overview of the data and are:

- normalized value of the concentration of internal standard for each samples analyzed calculate as:

$$||C_{ISTD}|| = \frac{C_{ISTD}}{< C_{ISTD} >} \tag{3.7}$$

  where the C_{ISTD} is the concentration of the internal standard in that samples and $< C_{ISTD} >$ is the mean value of the concentration of internal standard over a triplication of experiments.

- normalized value of area of the target compound, calculate as:

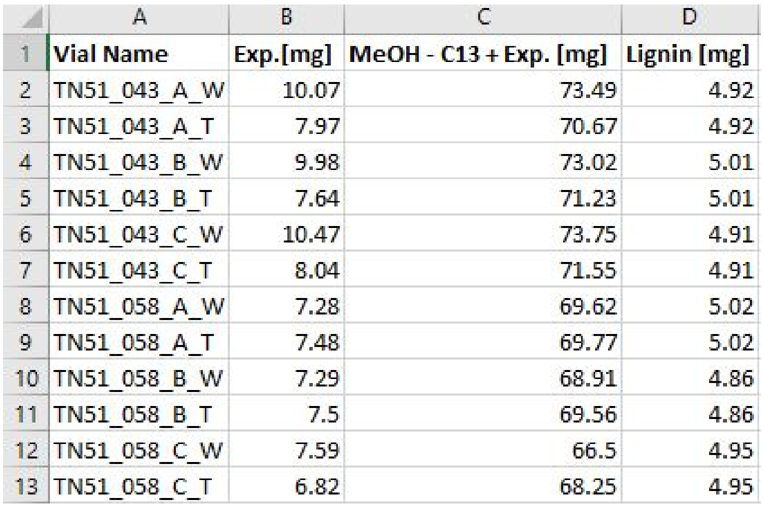
$$||A_x|| = \frac{A_x}{A_{ISTD}} \frac{1}{||C_{ISTD}||} \tag{3.8}$$

where A_x is the area of the target compound, A_{ISTD} is the area of the internal and $||C_{ISTD}||$ is the normalized concentration of internal standard calculated as shown in the previous equation.

The results produced with the LC-MS were handled by Xcalibur and summarized into CSV files. Data acquisition, data exploration and data processing were carried out with the help of MATLAB, while visualization is carried out within Origin Labs.

3.6.1 Matlab Code

Refer to Appendix B to see the MATLAB code used to read data from a previously created excel file, process and write the outcome to a new excel document. The working of the code is explained here below, please note that the code was tested and run on MATLAB R18b and its compatibility with previous releases is unidentified. To ease the process and correctly prepare the working environment, it is suggested to create a folder containing the MATLAB script, the Xcalibur CSVs and the "vial" file. Before starting, open the CSV file produced by Xcalibur, select the working sheet, copy its content and paste it onto an excel file. Save it in the same directory. The file "vials" contains information inherent how the vials prepared are located, in particular the amount of sample and the weighted amount of solution with MeOH and internal standard, as is shown in Fig.3.4. In addition it is listed the exact quantity of lignin added in each experiment. In order for this to work, the "vial" file must contain all the experiments name that are present in the file excel under analysis. Extra experiments and the order would not affect the outcome.



	A	B	C	D
1	Vial Name	Exp.[mg]	MeOH - C13 + Exp. [mg]	Lignin [mg]
2	TN51_043_A_W	10.07	73.49	4.92
3	TN51_043_A_T	7.97	70.67	4.92
4	TN51_043_B_W	9.98	73.02	5.01
5	TN51_043_B_T	7.64	71.23	5.01
6	TN51_043_C_W	10.47	73.75	4.91
7	TN51_043_C_T	8.04	71.55	4.91
8	TN51_058_A_W	7.28	69.62	5.02
9	TN51_058_A_T	7.48	69.77	5.02
10	TN51_058_B_W	7.29	68.91	4.86
11	TN51_058_B_T	7.5	69.56	4.86
12	TN51_058_C_W	7.59	66.5	4.95
13	TN51_058_C_T	6.82	68.25	4.95

Figure 3.4: Example of the file construction of the excel file "vials". The column "Exp." is the amount of the experiment inserted into the vials to be analyzed, the column "MeOH-C13+Exp" is the total mass present in the vial, which is the sum of the mass experiment and the mass of the stock solution of ^{13}C-Vanillin. The column "Lignin" is the amount of lignin used during the experiment.

Once the files ready, execute the code. The following events will be initialized.

1. Open and read the excel and "vials" files, compare the sample names and prepare a new cell structure.

2. Separate between polar and apolar samples and sort the data in group of triplicated sample.

3. perform different calculations on the data

4. Calculate the real dilution factor for each vials and the real concentration of internal standard.

5. Calculate the average concentration of internal standard

6. Calculate a normalized concentration of the internal standard (the real amount of internal standard present the vials analyzed divided by the average concentration of internal standard calculated at the previous point).

7. Calculate the normalized area for each experiment: the area is normalized respect to the area of the internal standard and to the normalized concentration of the internal standard.

8. Calculate the average normalized area for a triplication of experiments and the Cv values.

9. Generate a cell structure DataFinal where you can find:

   - Name of each experiment;
   - All the information present in the file “vials”;
   - The real concentration of internal standard in each sample and the average value;
   - The dilution factors for each sample analyzed with Xcalibur;
   - The areas of the target compound and of the internal standard calculated by the software Xcalibur for each sample analyzed ;
   - The normalized concentrations of internal standard for each sample analyzed with Xcalibur;
   - The normalized area of the target compound for each sample analyzed;
   - The average value of the normalized area of the target compound and CV value for each triplication of the experiments.

10. Generate a cell structure”DataFinal_02” where you can find

    - Name of each experiment;
    - All the information present in the file “vials”;
    - The real concentration of internal standard in each sample and the average value;
    - The dilution factors for each sample analyzed with Xcalibur;
    - The areas of the target compound and of the internal standard calculated by the software Xcalibur for each sample analyzed;

The current version of the code requires that the results in DataFinal and DataFinal_2 are manually copied and pasted on an excel file to further analysis. It would be possible to automate this part but the time required to implement the change would make the operation non-beneficial. It would be possible to implement the possibility of reading multiple excel files at a time and even create a sort of GUI using MATLAB GUIDE to allow the user to select the working mode. However, to make the code efficient, it would be better to create sort of a database (like an excel file) where the name of the samples already analyzed are stored. By matching the names in the database to the sheets names of the analyzed excel, it would be possible to avoid to re-analyze the same samples and improve the performance. Moreover, it would be necessary to integrate a way to choose if a sheet has to be re-analyzed and a way to manually delete old sheets (these two could be done manually by deleting their names from the “database” file). The code is built in a way to work with the sample naming methodology used in this thesis (ShortName_number-of-experiment_A/B/C), as explained in section 3.3. The code has to be adapted whenever the sample names structure is changed.

4 Results

This research is based on two main hypotheses, i.e, the biphasic lignin depolymerization in a biphasic water - organic solvent in the presence of polyoxometalates as catalysts. POMs are a class of anionic metal-oxo clusters constructed mainly by early transition metals in high oxidation states. They are characterized to be strong acids with very high Brønsted and Lewis acidity [11]. They have found application in the fractionation of lignocellulosic biomass as an alternative for processes free of sulphur or chlorine. In fact, they allow the oxidation of lignin and the reduced POM can be regenerated in the presence of an appropriate oxidizing agent, as described by the following reactions:

$$POM_{(ox)} + Lignin \rightarrow POM_{(red)} + Lignin_{(ox)} \tag{4.1}$$

$$POM_{(red)} + O_2 \rightarrow POM_{(ox)} + H_2O \tag{4.2}$$

In order to ensure the reactions 4.1 and 4.2 occur, the oxidation potential of the POMs must be greater than that of lignin but at the same time lower than that of the oxidizing agent, namely:

$$^{\circ}E_{Lignin} > {}^{\circ}E_{POM} > {}^{\circ}E_{O_2} \tag{4.3}$$

where $^{\circ}E_{\mathrm{O_2}}$, $^{\circ}E_{\mathrm{POM}}$,$^{\circ}E_{\mathrm{O_2}}$ are respectively the oxidative potentials of lignin, polyoxometalates and the oxidizing agent found. In literature it is possible to find values for oxidative potentials, for lignin it is equal to 0.4-0.6 eV vs. NHE at pH 1 [11, 12, 13], for POM 0.68-0.71 eV vs. NHE at pH 1 [11, 13, 14], and for oxygen 1.22 eV vs. NHE at pH 0.059 [11].

In this research it was decided to investigate the effectiveness of POMs in the conversion of lignin into low molecular weight aromatics. In addition, studies with model compounds have shown that POMs can be suitable candidates as catalysts for lignin depolymerization [48] and one study applied POM-Mo in the presence of MeOH obtaining a maximum reaction yield of 5.18 wt.%. The other hypothesis underlying this thesis is based on the construction of a biphasic system for lignin depolymerization in line with the study previously published by Bjelić et al. [9]. Their research highlighted the potential for depolymerization of Kraft lignin in a biphasic water-octanol system in the presence of sulfuric acid at 170°C . The reaction occurs at the water-octanol interface: the polar phase is the reactive phase while the apolar phase allows the protection of monomeric products from condensation and repolymerization reactions. In fact, experiments in biphasic environment allowed a vanillin yield six times higher than the yield obtained by the experiment carried out in aqueous medium (the yields are respectively 1.11 wt.% and 0.17wt.%). These results demonstrate the effectiveness of biphasic depolymerization of lignin.

The design of a biphasic system for lignin depolymerization is influenced by a series of parameters such as catalyst types and concentrations, solvents, temperature, but is also influenced by the geometry and structure of the reactor used. In this thesis the variation of the parameters has been conducted in order to select with each series of experiments the best conditions to investigate the possible potentialities of the system. The study is carried out on two different POMs and the effect of their concentration on the oxidation of lignin is investigated. The experiments were performed both in inert and oxidative atmospheres to understand the influence of oxygen on the reaction. Due to the acidic behaviour of POMs and therefore low pH that characterizes aqueous solutions with POMs, it is necessary to investigate whether the reaction yields are due to acidolysis or oxidation: for this scope blanks experiments with H_2SO_4 as catalyst have been analyzed. At the end of this set of experiments the catalyst that provides better yields will be object of further investigation, such as temperature investigation and variation of parameters related to the biphasic system.

4.1 Solvent screening

The design of an efficient biphasic system for lignin depolymerization falls primarily within the choice of the apolar solvent. Four different systems have been studied, as shown in table 4.1. All biphasic experiments are conducted in a 50:50 polar:apolar system with a lignin concentration of 1 mg/ml. Tert-butanol and acetonitrile are miscible with water, therefore to guarantee the presence of two phases, water was saturated with NaCl. The presence of salt affects its surface tension and decreases the miscibility of the two phases.

Solvents	**Catalyst [muM]**	**Gas**	**Temperature [°C]**
Water - Octanol	POM-Mo (10)	Ar	170
Water - Toluene	POM-Mo (10)	Ar	170
Salty Water[a] - Acetonitrile	POM-Mo (10)	Ar	170
Salty Water[a]- Tert-Butanol	POM-Mo (10)	Ar	170

[a] Salty water means Milli-Q water saturated with NaCl.

Table 4.1: List of the biphasic systems under analysis. Each experiment was performed starting with 1 mg/ml of Kraft lignin and a reaction time of 20 minutes.

Tert-butanol did not prove to be a suitable solvent for our system: the experiment was stopped due to pressure rise above the maximum operating limit of 30 bar. In addition, tert-butanol was almost completely consumed as it was oxidized by the catalyst (POM-Mo). The other three systems were compared according to three evaluation criteria: solubility of the target compounds, stability and greenness of the solvent. Stability means that the solvent does not react with the reagent system and is not consumed in the course of the reaction. Analysis with Compound Discover showed that octanol is not completely stable. Moreover, in the studied conducted with octanol and water by Bjelić et al. has been noted that the octanol act as capping agent. All three systems resulted in more or less similar vanillin yields : 0.83 wt.%, 0.98 wt.%sw-o and 1.06 wt.%, respectively for the system water-octanol, salty water-acetonitrile and water- toluene. Furthermore, the solubility was taken into account by considering the vanillin partition coefficient K_D calculated as the ratio between the concentration of vanillin in the apolar and polar phases. The respective K_D are 23.4 for octanol, 5.6 for toluene and 11 for acetonitrile. It is surprising to note that the solvent with the lowest partition coefficient gives a higher yield in terms of vanillin produced. In the paper of Capello, C et al. [49], 26 organic solvents were compared to generate a ”green” score. This score is based on the combination of two methods: the life-cycle assessment (LCA) method which gives the quantification of emissions and resource use over the whole life-cycle of a solvent; and the EHS method that identifies the potential substance hazards. Toluene results "greener" than acetonitrile under both methods. Acetonitrile and toluene have different strengths that could make them feasible candidates for lignin depolymerization. In this thesis has been decided to investigate the potentialities of toluene as solvent for the biphasic lignin depolymerization.

4.2 Catalysts screening

The concentration of the catalyst influences the depolymerization yield in a water-toluene biphasic system, the POMs, in absence of O_2 act as stoichiometric oxidizers. POMs have an acidic character, hence by increasing their concentrations, the pH of the system decreases. In order to understand whether the reaction yields are due to the concentration of the catalyst either to the low pH it has been necessary to run blank experiment with H_2SO_4 as catalyst. The concentration of H_2SO_4 used is equal to 150mM, which means a pH=0.5.

It is necessary to elucidate whether the catalyst attacks the liquid phase: for this reason experiments without lignin, in presence of catalyst and water-toluene have been performed. The results have showed that the toluene is not consumed.

The main results of this section are shown in Fig.4.1. The screening parameter used in this case was only vanillin yield, as it was observed that it is the most abundant reaction product.

The first conclusion is that the observed vanillin yield is considerably higher in biphasic systems than in monophasic ones. This is valid for the three catalytic systems: H_2SO_4, POM-Mo and POM-W. This result confirms the hypothesis that performing lignin depolymerization in a biphasic system provides an higher vanillin yield. It is highly possible that the presence of the second phase allow the protection of the monomeric products to side reaction and repolymerization, and moreover the continuous extraction from the water phase of the product allow to shift the depolymerization reaction towards the products (Le Chateliers' principle). The POM-W based system has provided low vanillin yields both in the presence of oxygen and in the presence of argon: the vanillin yield in both cases is about 0.4 wt.%. Moreover, it can be observed that the yield does not vary according to the amount of catalyst. There are two possible explanations for this trend: either this catalyst has not reached its activation temperature, or it is unable to break the ether bonds (in particular β-O4) and C-C linkages (which are highly difficult to break down) in the lignin structure. In literature, it can be seen that POM-W based is used as a heterogeneous catalyst for example dehydration of ethanol at temperatures above 250°C [50]. However, it is a completely different system and it is difficult to say whether the low activity of POM-W based can be attributed to the temperature. An idea to try to answer this question would be to repeat the reaction for different temperatures and observe the effect on vanillin yield. It has to be kept in mind that working at high temperatures is of low interest in a concept of biomass reuse.

In the case of H_2SO_4 some interesting results were found: the biphasic water-toleune system in the presence of O_2 provided a vanillin yield of about 0.4 wt.% while in Ar atmosphere a yield of approximately 1.6 wt.% was achieved, which means more or less quadruple. This result opens many questions of scientific interest and should be subject to more studies and in particular how the activity of H_2SO_4 and the system is affected by the presence of oxygen.

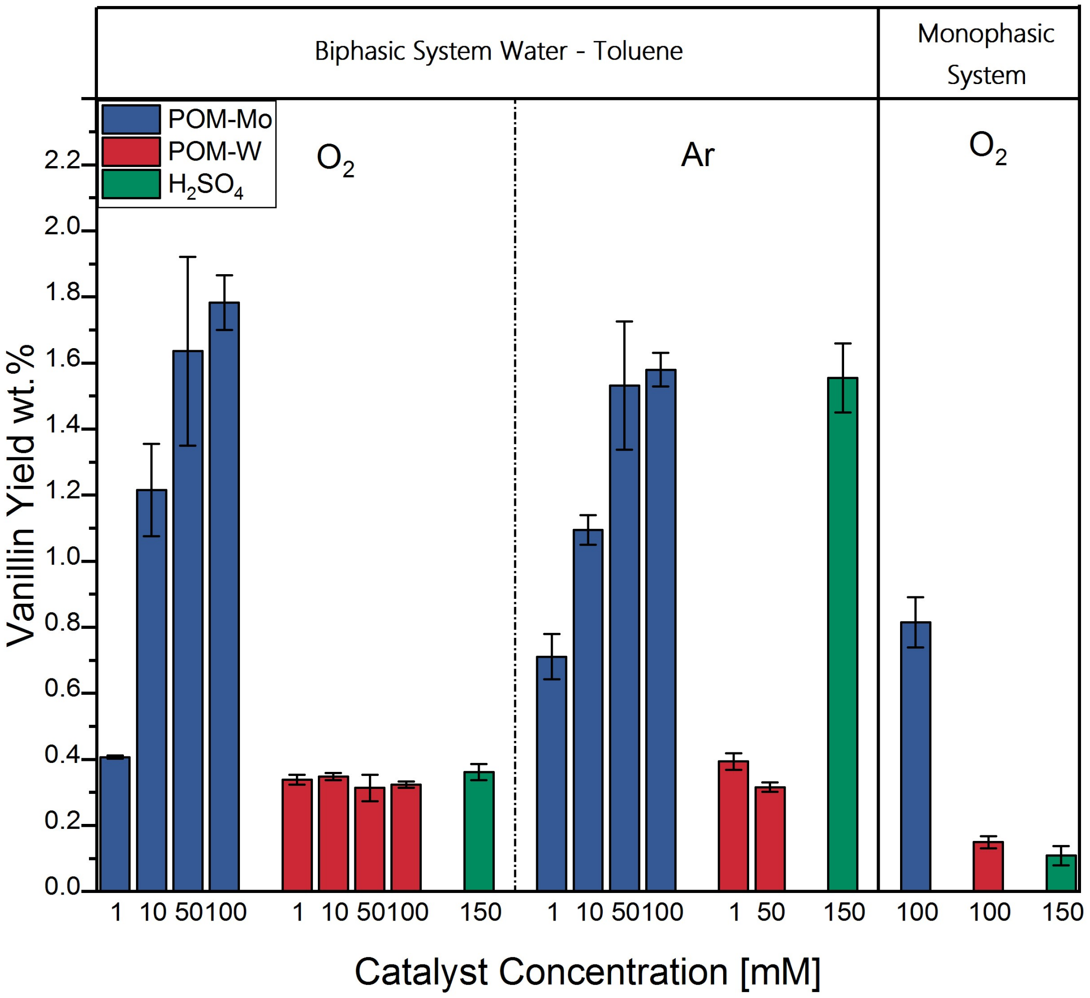


Figure 4.1: Representation of the vanillin yield of a series of biphasic and monophasic experiments (on the left and right of the solid line respectively) conducted in presence of 1mg/ml of Kraft lignin and heated up at 170°C for 20 minutes. The x axis represents the concentration of the catalyst used, whereas the y axis is the vanillin yield base on dry lignin content.POM-Mo is the abbreviation for the polyoxometalate $H_3PMo_{12}O_{40}$; POM-W is the abbreviation for the polyoxometalate $H_4Si_4W_{12}O_{40}$.

POM-Mo has shown to be the best candidate for lignin depolymerization at 170°C: both in argon and oxygen atmosphere as the concentration of POM-Mo increases, the vanillin yield increase and stabilizes at 50 mM (note how error bars in the Fig.4.1 at 50 mM overlap with those at 100 mM). In theory POM-Mo in the presence of oxygen should be able to regenerate and therefore an obvious expectation would be to see the regeneration of the catalyst and a higher depolymerization yield under O_2 atmosphere. In the case of these results there is no appreciable difference in terms of vanillin yield between experiments under Ar or O_2 atmosphere: this means that it is highly likely that catalyst regeneration will not occur in our system due to the low oxygen concentration. In fact, as better highlighted in Tab.4.2, similar yields are obtained at the same catalyst concentrations under the two different atmospheres.

It is therefore interesting to compare the amount of oxygen present in the system at room temperature and atmospheric pressure to the number of theoretical lignin monomeric units and the concentration of POM-Mo. The theoretical monomeric units in softwood Kraft lignin can be estimated assuming that lignin is mainly made of G units (97% expressed as molar percentage)[10]. The G unit is the 4-(3-hydroxyprop-1-en-1-yl)-2-

POM-Mo [mM]	Gas	Vanillin Yield wt.%	Standard Deviation
1	O_2	0.41	0.05
1	Ar	0.71	0.07
10	O_2	1.22	0.14
10	Ar	1.09	0.05
50	O_2	1.64	0.29
50	Ar	1.53	0.19
100	O_2	1.78	0.08
100	Ar	1.57	0.05

Table 4.2: Vanillin yield comparison of the experiments conducted in presence of POM-Mo at different concentration in O_2 or Ar atmosphere. The vanillin yield presented are the mean value over a triplication of experiments. All these experiments have been conducted with 1 mg/ml of Kraft lignin and heated for 20 minutes at 170°C.

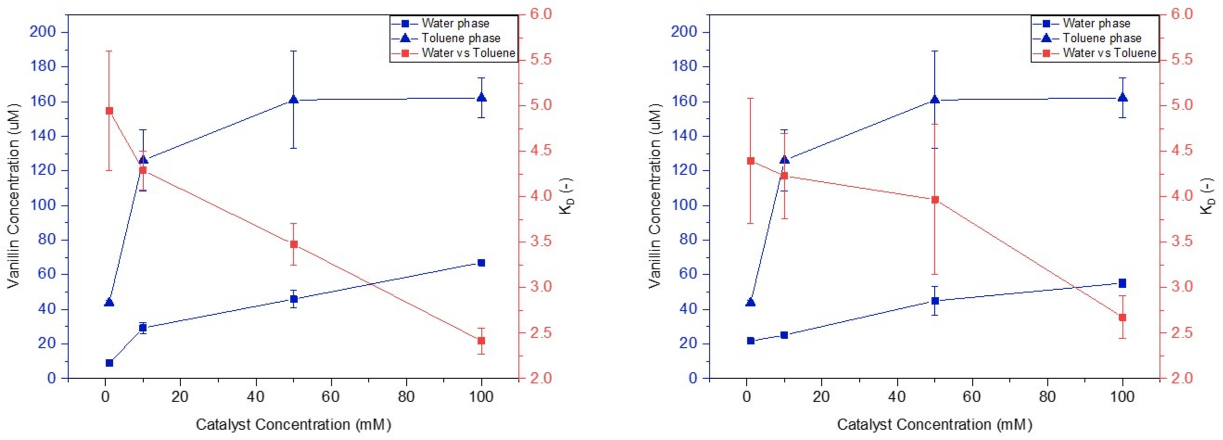
methoxyphenol, also known as coniferyl alcohol and it has a molar mass of 180.2 g/mol. The number of lignin monomeric units does not change from one experiment to the other as all experiments has been conducted with 1 mg/ml of Kraft lignin, and this means 5 mg of Kraft lignin. For the oxygen quantification, it is necessary to quantify the amount of O_2 in the head-space of microwave batch reactor vial, in the toluene and in the water phases. The calculations have been done assuming that in the head space there is only O_2 and that both phases have reached the saturation as pure liquids. The number of moles of O_2 is estimated at the conditions of the filling gas during the experiment preparation, which are 1 atm and 20°C. This procedure is explained in section 3.4. First, by using the ideal gas law it is possible to approximate the moles in the head-space. The moles of oxygen dissolved into the toluene phase are calculated using Henry's equation: the Henry's constant for the solubility of oxygen in the toluene phase was taken from the literature[51], its value is $H_{293.2K} = 10670(Pam^3)/mol$. The solubility coefficient of oxygen in water was taken from the literature [52], its value is $S_{293.2K} = 0.00125 mol/dm^3$. The results of these calculation are summarized in Tab.4.3.

It is necessary to underline that the amount of oxygen present in the system is calculated at room temperature and pressure whilst our final temperature is 170°C for these series of the experiment. The solubility of oxygen in the two solvents decreases as the temperature increases, hence the amount of dissolved oxygen in the two phases will be lower at 170°C. Moreover, a study conducted on the depolymerization of softwood Kraft lignin in presence of 50mM of POM-Mo showed the presence of 10 bar of oxygen allows the regeneration of the catalyst: they obtained a vanillin yield of 2.45 wt.% in presence of O_2 respect to 1.84 wt.% under N_2 atmosphere [17]. It is clear that the microwave batch reactor is not the right set-up to investigate the regeneration of the catalyst both for fluidodynamics aspects and its limit operation pressure. For this purpose, it was decided to continue the study of the biphasic depolymerization of Kraft lignin in a microwave reactor in presence of POM-Mo under Ar atmosphere and with a catalyst concentration of 50mM, which means that are available around two moles of catalyst per lignin building blocks.

Catalyst [mM]	Catalyst [μmol]	Lignin Building blocks [μmol]	O_2 Head-space [μmol]	O_2 Total [μmol]
100	250	28	208	235
50	125	28	208	235
10	25	28	208	235
1	2.5	28	208	235

Table 4.3: Comparison between the amount of catalyst, lignin building blocks and O_2 present in the microwave batch reactor.The number of lignin building blocks is calculated considering G units as the principal monomeric unit in softwood Kraft lignin and considering 5 mg of lignin. The amount of catalyst is calculated based on the concentration used in the experiments. The amount of oxygen is calculated at T= 20 °C and P=1 atm. The concentration of O_2 present in the two phases is respectively 24 μmol for toluene and 3 μmol for water.

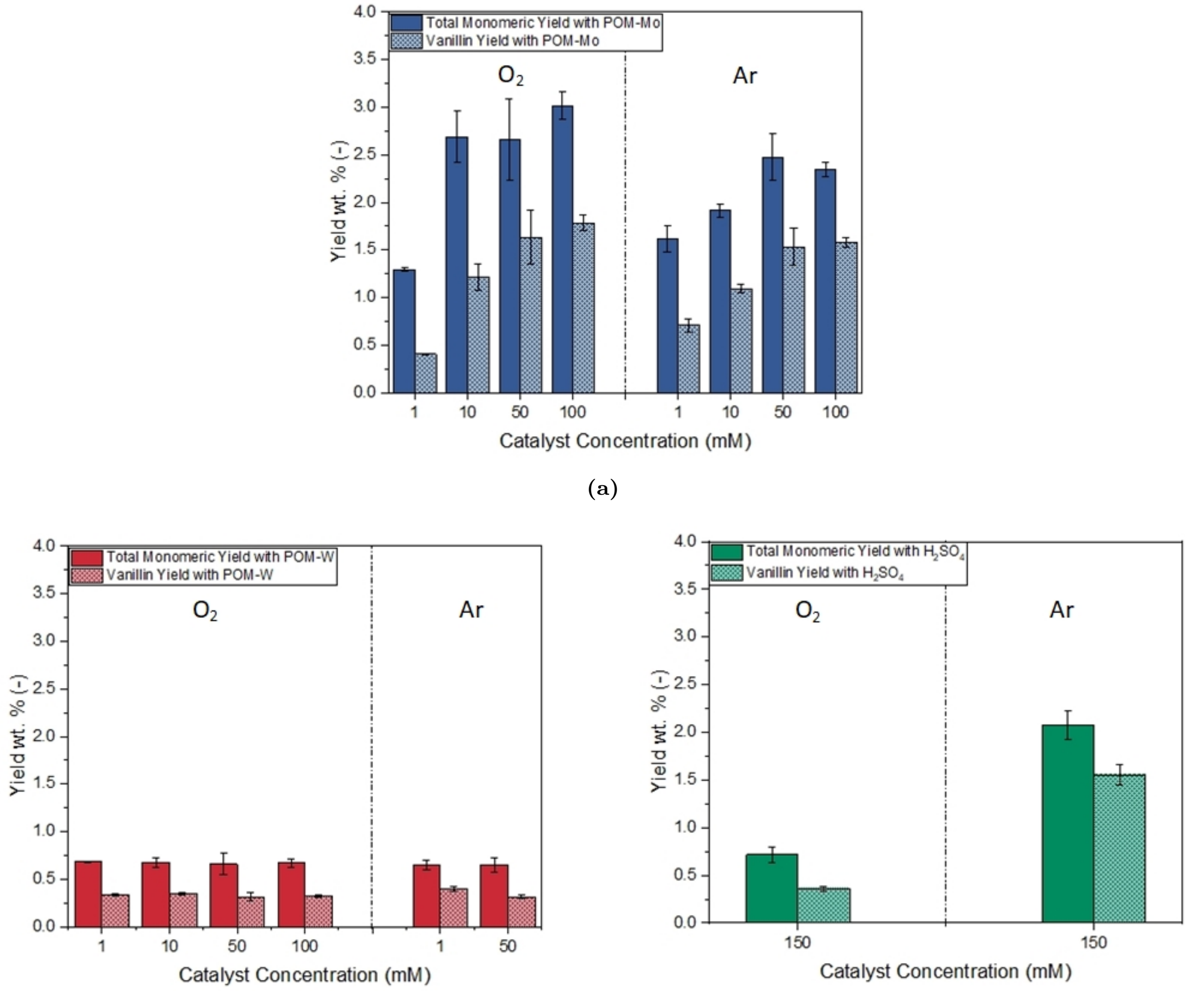
Biphasic system for lignin depolymerization should guarantee that the reaction products are present in greater amount in the apolar phase for the motivations explained before. Therefore, it is very interesting to analyze the trend of the distribution coefficient K_D, calculated as the ratio between the concentration in the organic and the polar phases, as function of the POM-Mo based concentration. Fig.4.2 shows how the trends of the vanillin concentration in the water and toluene phases and the K_D of vanillin versus the concentration of POM-Mo: as the concentration of POM-Mo increases, the amount of vanillin present in the apolar phase and its K_D decrease both under Ar and O_2 atmosphere. A possible explanation is that POM-Mo stabilizes vanillin in the polar phase.



(a) **(b)**

Figure 4.2: Influence of POM-Mo on the concentration of vanillin in the toluene and water phase. The graphs are characterized by double y axis: on the left is the vanillin concentration, whereas on the right side is the K_D, partition distribution coefficient. The x axis represents the concentration of POM-Mo.The blue triangles represent the concentration of vanillin on the toluene phase, and the blue square ones represent the concentration of vanillin in the water phase. These experiments have performed with 1 mg/ml of Kraft lignin at 170°C for 20 minutes under (a) O_2 atmosphere or (b) Ar atmosphere.

In addition to vanillin, other aromatic compounds have been identified as reaction products of lignin depolymerization. The complete list of identified products can be found in section 3.6. Particularly surprising is the production of syringaldehyde: softwood Kraft lignin is almost exclusively composed of G units and it would be expected to obtain syringaldehyde from lignin in which there is a certain percentage of S units. Fig.4.3 shows the yields of all identified compounds compared to the vanillin yields for the three catalysts POM-Mo, POM-W and H_2SO_4.



(a)

(b)

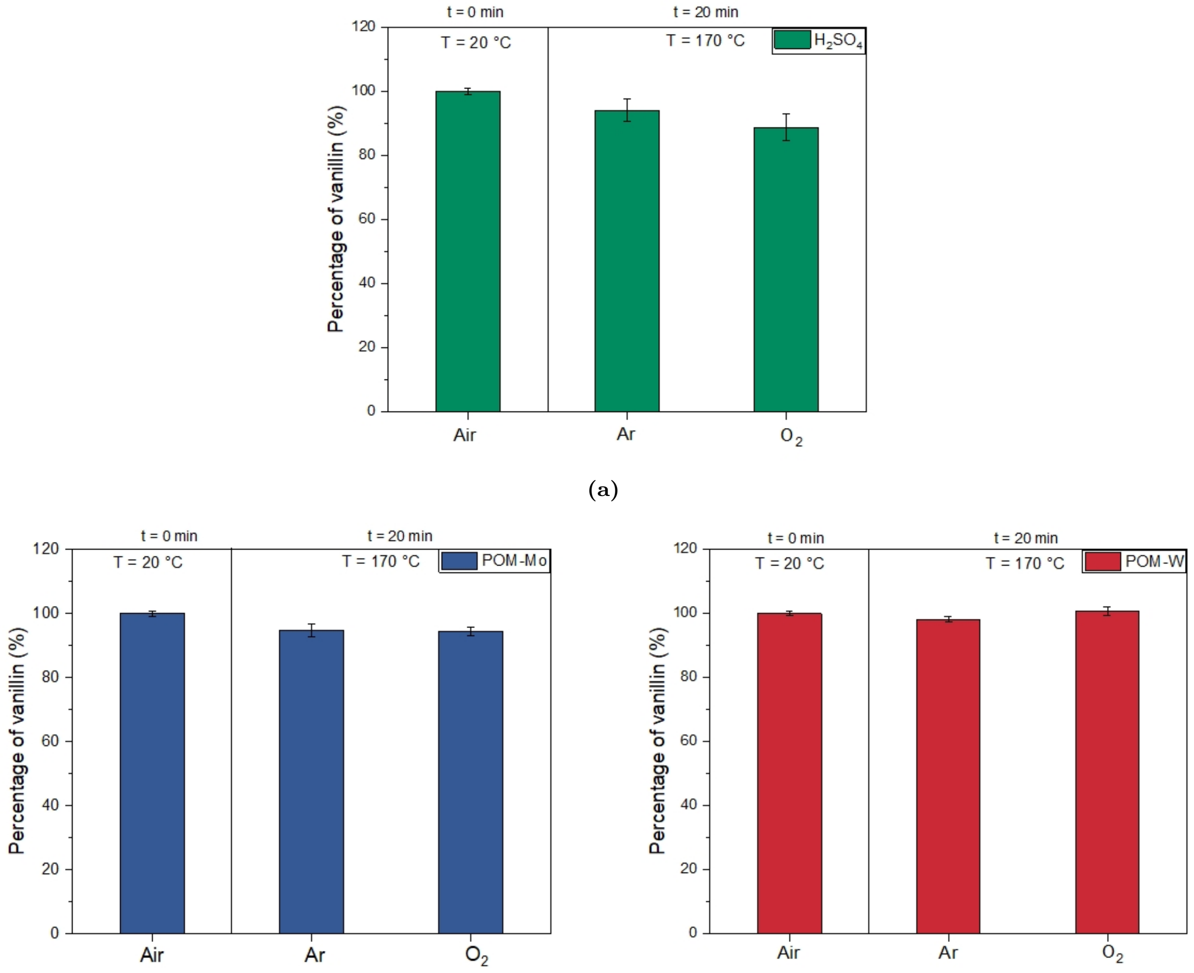
(c)

Figure 4.3: Yield of all the monomeric compounds identified and vanillin yield in water-toluene biphasic experiments in O_2/Ar atmosphere respect to the catalyst concentration, in particular (a)POM-Mo, (b) POM-W and (c) H_2SO_4. The experiments have been conducted with 1 mg/ml of Kraft lignin at 170°C for 20 minutes.

As can be seen, the general trend has not changed: the system in presence of POM-Mo is the one that provides the best yields having an average total yield value of 3.02% in presence of O_2 and a catalyst concentration of 100mM, of which 1.78% is vanillin. However, it can be noted that the error bars overlap between 50 and 100 mM of POM-Mo in O_2 and for this reason it was desired to continue the study of the system in the presence of a POM-Mo concentration of 50mM. Furthermore, it can be seen in Fig.4.3 that using POM-W or H_2SO_4 result in lower yields of the other monomeric compounds identified compared to yield obtained with POM-Mo.

4.3 Evaluation of vanillin stability

In this section the aim is to investigate whether vanillin, which is the major monomeric product as shown in the section 4.2, is subjected to degradation/repolymerization reactions over the reaction time. This study was carried out by performing biphasic water-toluene experiments with vanillin as the starting material instead of lignin. Data analysis was carried out by analyzing the amount of vanillin present at zero time (i.e. before replacing the atmosphere inside the reaction vial) which corresponds to 100% vanillin, to the amount of vanillin present at the end of the reaction. The results are shown in Fig.4.4 for POM-Mo, POM-W and H_2SO_4 both in O_2/Ar atmosphere.



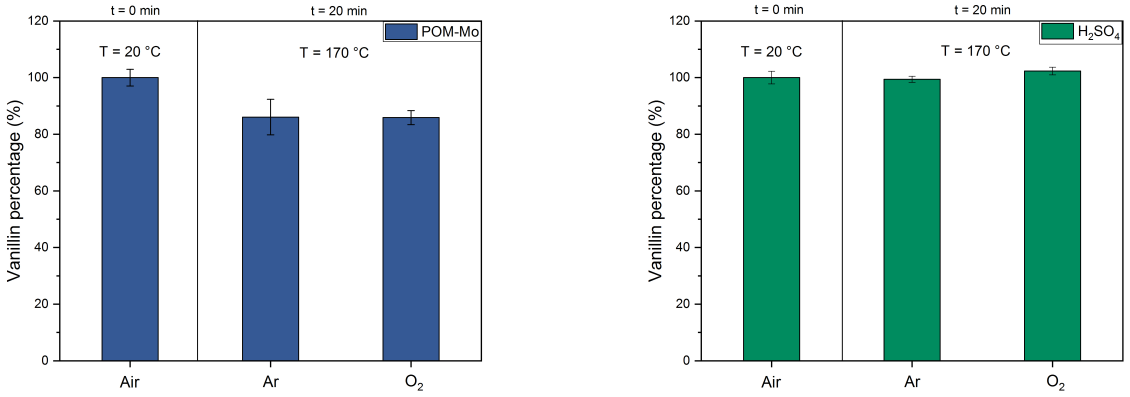
(a)

(b)

(c)

Figure 4.4: Variation of vanillin over the reaction time in a water-toluene biphasic system under O_2/Ar atmosphere with an initial vanillin concentration of 100μM in presence of: (a) 150 mM of H_2SO_4, (b) 50 mM of POM-Mo and (c) 50mM of POM-W. The reactions were run at 170° for 20 minutes.

Monophasic systems were tested using the same technique in order to determine the effect of the second phase on the loss of vanillin, which are shown in Fig.4.5.



(a) **(b)**

Figure 4.5: Variation of vanillin over the reaction time in monophasic system under O_2/Ar atmosphere with an initial vanillin concentration of 100μM in presence of: (a) 50 mM of POM-Mo, (b) 150 mM of H_2SO_4.The reactions were run at 170° for 20 minutes.

The Tab.4.1 summarizes all the results in terms of average vanillin loss both biphasic monophasic experiments.

Catalyst	**Catalyst Concentration** [mM]	**Phases**	**Gas**	**Vanillin Loss** Average %
H_2SO_4	150	Water-Toluene	O_2	11.82
H_2SO_4	150	Water-Toluene	Ar	5.83
H_2SO_4	150	Water	O_2	0
H_2SO_4	150	Water	Ar	0.56
POM−Mo	50	Water-Toluene	O_2	5.27
POM−Mo	50	Water-Toluene	Ar	5.24
POM−Mo	50	Water	O_2	14.11
POM−Mo	50	Water	Ar	13.95
POM−W	50	Water-Toluene	O_2	0
POM−W	50	Water-Toluene	Ar	0

Table 4.4: Average vanillin loss for experiments performed with a initial concentration of vanillin equal to 100μM at 170°C in presence of different catalysts.

The results show that in a biphasic environment the presence of oxygen almost doubles vanillin losses in the system in the presence of H_2SO_4. On the other hand, for the biphasic system in the presence of POM-Mo, there are no particular differences in the loss, which remain modest (about 5%). As can be seen from the Tab.4.1, the presence of the second phase allows the protection of vanillin from degradation only for the system in presence of POM-Mo: it goes from a loss of 5% in biphasic environment to about 14% in monophasic environment, which is more than two and a half times both in oxidative and inert atmosphere. On the other hand, higher vanillin losses are reached in biphasic system compared to the monophasic in presence of H_2SO_4 as catalyst. As already discussed, the POM-W catalyst showed poor results in lignin depolymerization: its low activity is confirmed by the absence of vanillin loss.

4.4 Temperature investigation of POM-Mo

The results presented in this paragraph are intended to show whether the temperature can be a key parameter for the reaction yield in presence of POM-Mo.

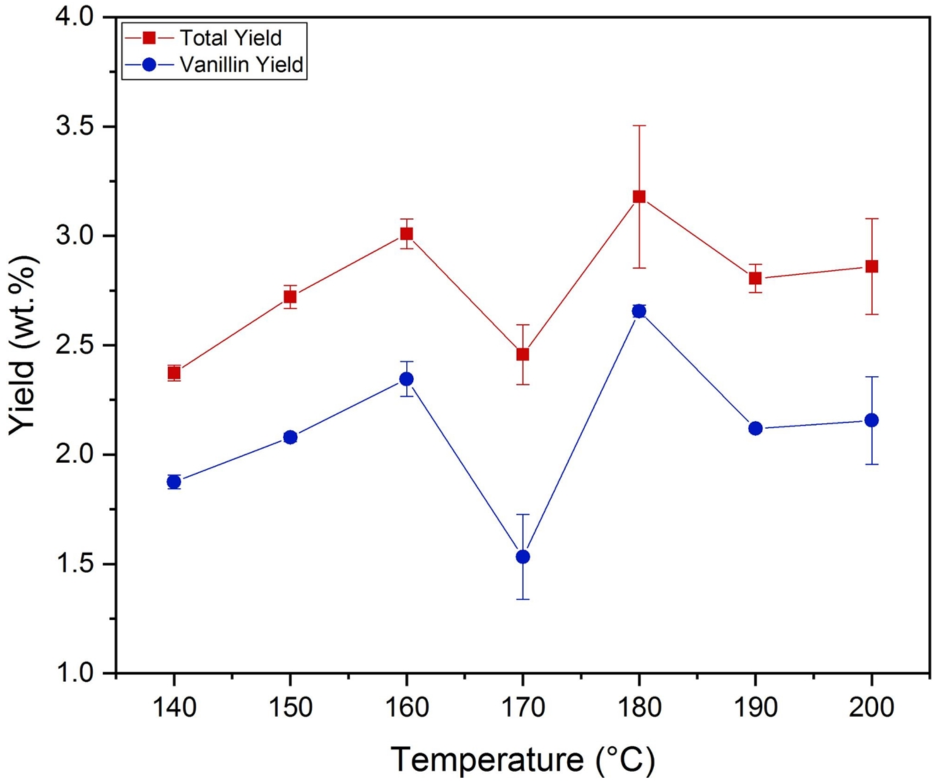


Figure 4.6: Influence of temperature on the reaction yield. In red the total monomeric yield, by mean of the compound identified, in blue vanillin yield. The experiments were performed in water-toluene biphasic system with 1 mg/ml of Kraft lignin in presence of 50 mM of POM-Mo under Ar atmosphere.

Fig.4.6 shows the total monomeric reaction yield and the vanillin yield as a function of temperature. Both yields increase with an increment in the temperature up to 160 °C to then decrease at 170 °C. At 180°C the highest averaged yields were observed, respectively 3.19 wt.% for the otal and 2.16 wt.% for the vanillin. After 180 °C, both yields decrease. A plausible explanation for the lower yields at 170°C lies in one property of lignin: the glass transition temperature of softwood Kraft lignin has been reported in the literature to be 160-170°C [15, 16]. Since the transition state is an endothermic process, it is possible to hypothesize that lignin ”subtracts” available heat from the catalyst to perform the transition state.

4.5 Experiments with MeOH

Experiments were carried out in presence of MeOH in order to investigate its effect on lignin depolymerization. The main hypothesis to be verify is whether MeOH allows radical alkylation [1]. An ETHZ research group carried out a study on the depolymerization of Kraft lignin in aqueous environment in the presence of POM-Mo and MeOH: their results showed that the presence of MeOH increase the yield only in O_2 atmosphere while in N_2 a decrease was observed [17, 18]. In addition, both in oxidative and inert atmospheres they have noted the production of a new compound in the presence of MeOH, namely methyl vanillate [17, 18].

On the basis of these hypotheses it was desired to investigate the role of MeOH in a biphasic system for lignin depolymerization. First of all, it was necessary to verify whether the water-toluene system could remain biphasic even in presence of MeOH: it was observed that the water-toluene system with 80 vol.% (with respect to the polar phase of 2.5 ml) of MeOH is biphasic at room temperature and pressure. It has been hypothesized that almost all MeOH is solubilized in the polar phase, based on literature studies on the ternary equilibrium of a water-MeOH-toluene system [53]. However, a significant difference was observed in the behaviour of lignin in the system: as the concentration of MeOH increased, lignin was more soluble and homogeneously dispersed in the polar phase. The concentration of MeOH used are 10, 20, 40, 60, 80 vol.% with reference to the volume of the polar phase. To perform the quantification of the reaction yield, as explained in section 3.6, it is necessary to use the densities of the two phases. In the case of these experiments the density of toluene was considered to be that of the pure substance, while the resulting densities of the various water-MeOH mixtures were calculated from data in the literature [21].

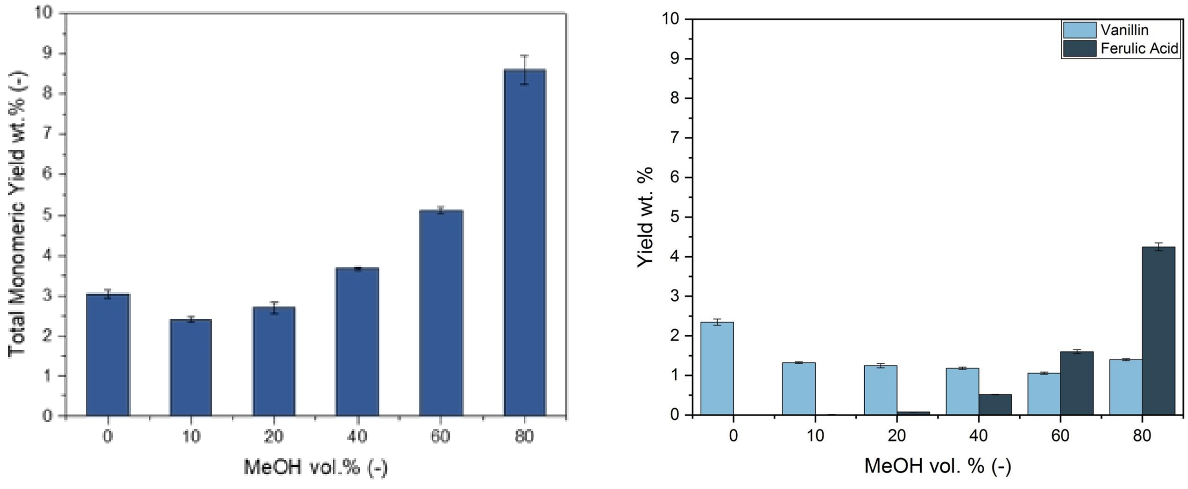
Water [vol.%]	**MeOH** [vol.%]	**Density** [mg/ml]
90	10	984.7
80	20	971
60	40	943.3
40	60	907.3
20	80	856.7

Table 4.5: Density of aqueous MeOH solutions [21].

The results obtained are very surprising, as they differ substantially from the above mentioned study. Fig.4.7 shows how the presence of MeOH affects the reaction yield: from 20 vol.% upwards the total monomeric yield increases substantially, reaching an average yield of 8.59 wt.% in the presence of 80 vol.% MeOH.The total yield increases by more than two and a half times compared to the system without MeOH.

Methyl vanillate has been identified as a reaction product in the presence of MeOH, but not in such high quantities as it has been found in other studies (the average maximum value obtained is 0.21 wt.% in the presence of 80 vol.% MeOH). On the other hand, ferulic acid has been identified as a reaction product in abundant quantities. Fig- 4.7b shows the

yields of vanillin and ferulic acid depending on the amount of MeOH: in the absence of MeOH ferulic acid is not produced, but as the quantity of the alcohol present increases the production of ferulic acid and the production of vanillin remains almost constant varying between 1 and 1.4 wt.%, compared to 2.35 wt.% produced in the absence of MeOH. Usually ferulic acid has often been obtained as a reaction product in the treatment of Kraft lignin with bacteria.
Although the results in terms of yield were surprising, unfortunately the presence of MeOH affects the solubility of the reaction products, which is detrimental to the effective designation of a biphasic system for lignin depolymerization.
Fig.4.8 shows the distribution coefficients of the two main reaction products, i.e. vanillin and ferulic acid, according to the percentage of MeOH present: the trend is very similar for both substances and the K_D decreases as the percentage of MeOH increases.



(a) (b)

Figure 4.7: Influence of MeOH concentration on (a) the total monomeric yield and (b) the vanillin and ferulic yields. The volume percentage of MeOH is referred to the volume of the polar phase. The experiments has been performed at 160°C for 20 minutes under Ar atmosphere in presence of 50mM of POM-Mo

The study already mentioned above developed by the ETHZ group studied the depolymerization of kraft lignin in the presence of POM-Mo 80 vol.% MeOH has reported yields of 5.18% under O_2 atmosphere and 1.29% under N_2 atmosphere. The results presented here showed a reaction yield of 8.59% in the presence of Ar and 80% of MeOH and a different selectivity in the reaction products has been observed, as previously outlined. It is therefore evident that the presence of toluene as a second phase in the system influenced the selectivity and led to higher reaction yields.

4.6 Effect of the presence of NaCl

The biphasic system in the presence of MeOH has shown excellent reaction yields, but, as already pointed out, it does not allow the full effectiveness of the biphasic system. In order to solve this problem, it was decided to reduce the solubility in the polar solution by simply adding salts: the desired purpose is to obtain the phenomenon of extraction by salting-out. In order not to influence the catalytic system, it was decided not to use salts made with transition metals and that at the same time had a fair solubility in the

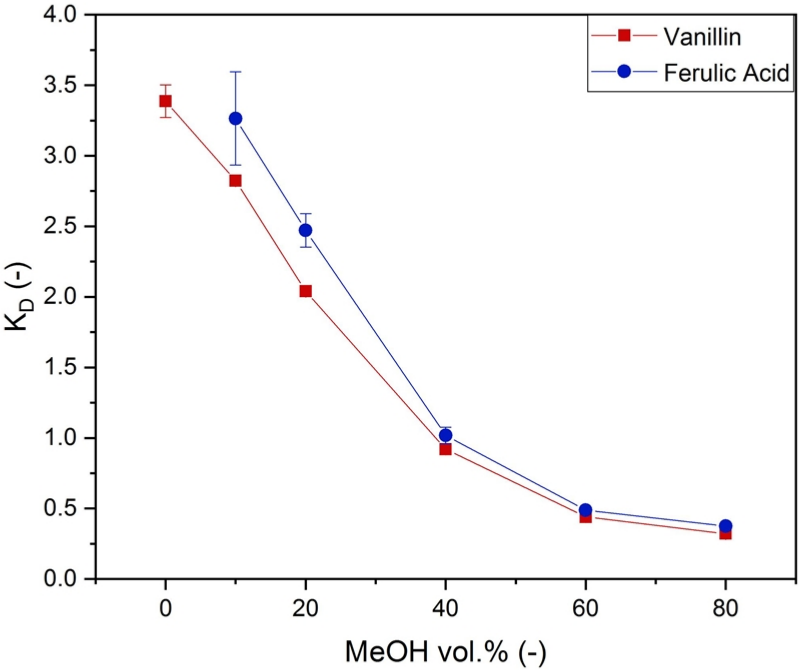


Figure 4.8: Variation of the distribution coefficient of vanillin and ferulic acid versus the concentration of MeOH. The concentration of MeOH is referred respect to the volume of the polar phase. These experiments have been conducted with 1 mg/ml of Kraft lignin, 50 mM of POM-Mo at 160°C for 20 minutes under Ar atmosphere.

water-MeOH solution. Therefore, NaCl was chosen as the salt as it has a low reactivity, reasonably solubility in the aqueous MeOH solution, easy-handling and low cost [19, 20]. The experiment was conducted in the presence of MeOH at 40 vol.% and by dissolving the polar phase 250mg of NaCl. This value was chosen based on 70% of the theoretical NaCl solubility in the polar phase. The solubility of NaCl used in the calculations is 2,665 mol/kg in a solution at 40 wt.% MeOH [20], which is equivalent to a solution at 46 vol.% MeOH considering a solution density of 943.3 mg/ml [21]. The results show that, as expected, an increase in yield and a decrease in the reaction products present in the polar phase compared to the corresponding experiment conducted in the absence of salt: the corresponding experiment in the absence of salt is characterized by a average yield of 3.67 wt.%, while the one in the presence of salt has an average yield of 4.48 wt.%, which means an increase of about 22 %. This increase in yield can be explained only by the phenomenon of salting out: in the polar phase the salt solvatation decreases the ”space” available for organic reaction products, which are subjected to the phenomenon of salting out in the apolar phase. In fact, the K_D values of vanillin and ferulic acid have increased respectively by 46 % and 69 %respect to the experiments conducted in absence of salt, obtaining a K_D of 1.34 for vanillin and 1.72 for ferulic acid. Allowing a better solubility of reaction products in the apolar phase, it can be seen that the presence of the second phase substantially affects the reaction yield, and therefore this result is also another confirmation of the effectiveness in the designation of a biphasic system for lignin depolymerization.

5 Conclusion

The research pursued in this thesis falls within the research of valid options for the substitution of fossil resources in the production of fine chemicals and the concept of 2nd generation biorefinery in order to carry out a total valorization of lignocellulosic biomass. Kraft lignin production covers more than 80 % of the world lignin production, so it would be scientifically relevant to find a method for the valorization of lignin produced by the most widespread process currently applied for lignocellulose fractionation.

In this thesis, an attempt was made to designate a biphasic system for depolymerization of kraft lignin into aromatic compounds. The biphasic system consists of two liquid phases, one apolar and the other polar: the idea in the designation of this system is to guarantee a reactive environment in the polar phase and the recovery of the solution products and their protection from deoplimerisation/degradation reactions through the presence of an apolar phase. The reaction products have a higher solubility in the apolar phase. The continuous removal of reaction products also tends to shift the reaction balance towards the products. In this research, a biphasic system consisting of water and toluene was investigated in the presence of POMs as catalysts.

The results showed that the biphasic system guarantees much higher reaction yields than the simple aqueous system, POM-Mo is the most promising catalyst in the screening performed. In addition, the presence of toluene has shown to provide protection to vanillin during the reaction time in presence of POM-Mo. Contrasting results were obtained in the presence of H_2SO_4: experiments conducted in the presence of vanillin as raw material have shown that the presence of toluene leads to a higher degradation of vanillin compared to the corresponding single-phase environment. This result, however, is possible to attribute to the inadequate choice of solvent in the presence of H_2SO_4: in fact, in the presence of H_2SO_4 and toluene sulfonation occurs, and consequently the idea of inert and protective environment no longer exists. Instead, in presence of POM-Mo it has been shown that the presence of toluene allows the protection of vanillin during the reaction time.

Another point that was investigated is how MeOH can influence lignin depolymerization yields in biphasic environments with POM-Mo. It was observed that the presence of MeOH influences the reaction yield considerably, in fact yields of 2.7% were obtained in the absence of MeOH and a yield of 8.6% in the presence of 80% MeOH. However, the presence of MeOH led to a variation in reaction selectivity, providing ferulic acid as the new main reaction product alongside vanillin. However, from the point of view of an ideal biphasic system it has been seen that the presence of MeOH leads to a clear disadvantage: as the concentration of MeOH increases the monomeric reaction yield but also increases the concentration of the main reaction products in the aqueous phase, reaching values of the distribution coefficients less than 1 for vanillin and ferulic acid. As a result, the system has been modified by attempting to separate the monomeric products through a salting out. The addition of sodium chloride increased the solubility of the aromatic reaction products in the apolar phase, resulting in a yield increase of 22%.

The experiments conducted in this thesis have shown that the biphasic system is a valid option to investigate for lignin depolymerization. However, it is still to be investigated what are the possible pathways for the formation of reaction products. In addition, it would be of particular interest to understand the explanation for the formation of ferulic acid in the presence of MeOH, as other studies conducted in the presence of MeOH and POM-Mo have shown methyl vanillin to be a new reaction product. Other solvent options should be investigated and other possible couplings with other catalysts should also be evaluated. The option to increase the effect of salting out separation remains extremely interesting, and therefore other salts could be investigated. Not least, it would be interesting to investigate how this system could work in the presence of other technical lignins.

Acknowledgement

Finally, I would like to express my gratitude to the Catalytic Process Engineering Group at PSI for giving me the opportunity to collaborate on this groundbreaking research and for helping me to grow both professionally and personally. In particular, I would like to thank Kasia, Sasa and Fredi who supervised me during my permanence at PSI. I will always remember Kasia, an extraordinary woman, driven by a strong scientific passion and solid core values who has constantly guided me to the right path, by sharing her knowledge and pushing me to build my curiosity about alternative energies and chemicals form lignin.

I would also like to thank Prof. Besaind for being my mentor and for supervising me in writing my thesis, which is always a tough time for a student.

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I think to have a special family, so I want to express my deep gratitude for their continuous help and support: my parents Paolo and Germana and my brothers and sister (Vittorio, Ruben and Egle).

And then a special thanks goes to Michael, who support me and give me every day the force to start a new challenge of my life.

A Appendix - Procedure for the Experiments Preparation

The aim of this appendix is to describe in detail the procedure by which the microwave batch reactor has been prepared before heating. This preparation phase differs according to the type of experiment carried out, since during this thesis various parameters have been varied. For each type of experiment it was possible to write a common procedure that always provides for the preparation of stock solutions. Almost all of the experiments shown in this thesis have been repeated three times and for each triplication of the same experiment were used different stock solutions, in order to achieve greater data reliability. Solvents and solutions are added to the system using micropipettes.

A.1 Biphasic experiments in presence of lignin and POM-Mo, solvents screening

This section provides a description on the procedure used for the preparation of the experiments for the solvents screening. The total volume of the liquid phase is 5 ml, of which half is polar and half is apolar phase. A stock solution of 50mM of POM-Mo in Milli-Q water has been prepared. The procedure for preparing the microwave reaction vial is :

- Take a clean vial, weight inside 5 mg of Kraft lignin and note the weight;
- Add 500μL of the stock solution with POM-Mo 50 mM in order to obtain a final concentration of 10 mM of catalyst in the polar phase;
- Add 2ml of Milli-Q water;
- Add 2.5ml of the desired organic solvent;
- Insert a magnetic stirrer and close the vial with the cap using the crimper;
- Mix it on the vortex mixer for few seconds.

For the experiments conducted with "Salty Water", instead of adding 2ml of Milli-Q water is added 2ml of Milli-Q water saturated with NaCl.

A.2 Biphasic experiments water-toluene in presence of lignin and catalysts

This section provides a description of the general procedure used for the preparation of water-toluene biphasic experiments with 1 mg/ml of Kraft lignin in the presence of catalysts. The total volume of the liquid phase is 5 ml, of which 2.5 ml is polar phase and 2.5 ml apolar phase. Different catalysts, in different concentrations, have been introduced to the system through stock solutions, which are:

- POM−Mo: stock solution of 100mM in Milli-Q water;
- POM−W: stock solution of 100mM in Milli-Q water;

- H_2SO_4: stock solution of 150mM in Milli-Q water.

Note that the concentration of the catalyst is always related to the volume of the polar phase.

The procedure for preparing the microwave reaction vial is as follows:

- Take a clean vial and weight inside it 5mg of Kraft lignin and note the weight;
- Add the desired amount of catalyst from the stock solution by calculating the volume to be added using the relation:

  $$V_{cat.,stock} = \frac{V_{polar} C_{cat.,reac.}}{C_{cat.,stock}} \tag{A.1}$$

  where $V_{polar} = 2.5ml$ is the total volume of the polar phase, $C_{cat.,reac.}$ is the desired concentration of the catalyst and $C_{cat.,stock}$ is the concentration of the stock solution;
- Add a volume of Milli-Q water equal to $V_{H_2O} = (2.5 - V_{cat.,stock})ml$;
- Add 2.5 ml of toluene;
- Insert a magnetic stirrer and close the vial with the cap using the crimper;
- Mix it on the vortex mixer for few seconds.

A.3 Monophasic experiments in presence of lignin and catalysts

This section provides a description of the general procedure used for the preparation of monophasic experiments with 1 mg/ml of Kraft lignin in the presence of catalysts. The total volume of the liquid phase is 5 ml. Different catalysts, in different concentrations, have been introduced to the system through stock solutions, which are:

- POM−Mo: stock solution of 100mM in Milli-Q water;
- POM−W: stock solution of 100mM in Milli-Q water;
- H_2SO_4: stock solution of 150mM in Milli-Q water.

Note that the concentration of the catalyst is always related to the volume of the polar phase. The procedure for preparing the microwave reaction vial is as follows:

- Take a clean vial and weigh inside it 5mg of Kraft lignin and mark the exact weight;
- Add the desired amount of catalyst from the stock solution by calculating the volume to be added using the relation:

  $$V_{cat.,stock} = \frac{V_{polar} C_{cat.,reac.}}{C_{cat.,stock}} \tag{A.2}$$

  where $V_{polar} = 5ml$ is the total volume of the polar phase, $C_{cat.,reac.}$ is the desired concentration of the catalyst and $C_{cat.,stock}$ is the concentration of the stock solution;
- Add a volume of Milli-Q water equal to $V_{H_2O} = (5 - V_{cat.,stock})ml$;
- Insert a magnetic stirrer and closed the vial with the cap using the crimper;
- Mix it on the vortex mixer for few seconds.

A.4 Biphasic experiments water-toluene in presence of vanillin

Experiments in a biphasic water-toluene environment with vanillin as the starting material were carried out in the presence of various catalysts at a fixed concentration ($[\mathrm{POM{-}Mo}] = 50mM$, $[\mathrm{POM{-}W}] = 50mM$, $[H_2SO_4] = 150mM$). The total volume of the liquid phase is 5 ml, of which 2.5 ml is polar phase and 2.5 ml apolar phase. The initial concentration of vanillin is 100 μM (referred to the total liquid volume). To carry out these series of experiments, the following stock solutions have been prepared:

- 50mM of POM−Mo in Milli-Q water;
- 50mM of POM−W in Milli-Q water;
- 150mM of H_2SO_4 in Milli-Q water;
- 200μM of vanillin in toluene.

Note that the concentration of the catalyst is always related to the volume of the polar phase. The procedure for preparing the microwave reaction vial is as follows:

- Take a clean vail and add 2.5 ml of the stock solution of the desired catalyst.
- Add 2.5 ml of the stock solution of the vanillin dissolved in toluene;
- Insert a magnetic stirrer and closed the vial with the cap using the crimper;
- Mix it on the vortex mixer for few seconds.

A.5 Monophasic experiments in presence of vanillin

Experiments in monophasic environment with vanillin as the starting material were carried out in the presence of various catalysts at a fixed concentration ($[\mathrm{POM{-}Mo}] = 50mM$, $[H_2SO_4] = 150mM$). The initial concentration of vanillin is 100μM. To carry out these series of experiments, the following stock solutions have been prepared:

- 100mM of POM−Mo in Milli-Q water;
- 300mM of H_2SO_4 in Milli-Q water;
- 200μM of vanillin in Milli-Q water.

The procedure for preparing the microwave reaction vial is as follows:

- Take a clean vial and add the 2.5ml of catalyst from the stock solution;
- Add 2.5ml of stock soluion with vanillin;
- Insert a magnetic stirrer and closed the vial with the cap using the crimper;
- Mix it on the vortex mixer for few seconds.

A.6 Biphasic experiments water-toluene in presence of lignin, POM-Mo and MeOH

This section provides a description of the general procedure used for the preparation of water/methanol-toluene biphasic experiments with 1 mg/ml of Kraft lignin in the presence of POM−Mo at a fixed concentration of 50mM. The total volume of the liquid phase is 5 ml, of which 2.5 ml is polar phase and 2.5 ml apolar phase. Note that the amount of methanol added to the system and the catalyst concentration is related to the volume of the polar phase. The maximum amount of methanol added is to 80% v/v. To carry out these series of experiments a stock solution of 250mM of POM-Mo in Milli-Q water has been prepared.

The procedure for preparing the microwave reaction vial is as follows:

- Take a clean vial and weight inside it 5mg of Kraft lignin and note the weight;
- Add 0.5ml of the stock solution with POM−Mo;
- Add the desired amount of methanol V_{MeOH} calculating as:

  $$V_{MeOH} = \frac{V_{polar} MeOH\%}{100} \tag{A.3}$$

  where $V_{polar} = 2.5ml$ is the total volume of the polar phase, $MeOH\%$ is the desired volume percentage of methanol;
- Add a volume of Milli-Q water equal to $V_{H_2O} = (2.5 - V_{MeOH} - 0.5)ml$;
- Add 2.5 ml of toluene;
- Insert a magnetic stirrer and closed the vial with the cap using the crimper;
- Mix it on the vortex mixer for few seconds.

A.7 Biphasic experiments water-toluene in presence of lignin, POM-Mo,MeOH and NaCl

This section provides a description of the general procedure used for the preparation of water/methanol-toluene biphasic experiments with 1 mg/ml of Kraft lignin in the presence of POM-Mo, methanol and NaCl. The total volume of the liquid phase is 5 ml, of which 2.5 ml is polar phase and 2.5 ml apolar phase. Note that the amount of methanol added to the system is related to the polar phase. The amount of methanol added is fixed to 40% v/v and the POM-Mo concentration to 50mM. Note that the amount of methanol added to the system, the catalyst and salt concentration is related to the volume of the polar phase.

To carry out these series of experiments, the following stock solutions have been prepared:

- 100mM of POM−Mo in Milli-Q water;
- 250mg/ml of NaCl in Milli-Q water.

The concentration of NaCl and catalyst are fixed in all these experiment respectively to 250mg/ml and 50mM. Note that the amount of methanol added to the system ,the catalyst and salt concentration is related to the volume of the polar phase. The procedure for preparing the microwave reaction vial is as follows:

- Take a clean vial and weight inside it 5mg of Kraft lignin and note the weight;
- Add 0.5ml of the stock solution with POM-Mo;
- Add 1 ml of methanol;
- Add 1ml of the stock solution with NaCl;
- Add 2.5 ml of toluene;
- Insert a magnetic stirrer and closed the vial with the cap using the crimper;
- Mix it on the vortex mixer for few seconds.

B Appendix - Matlab Code

```
clc; clear;

%% Section 1: Constants
% Here are listed all the constants necessary throughout the code

rhoW = 943.3;  % Density of Water [mg/ml]
rhoT = 862.3;  % Density of Toluene [mg/ml]
rhoM = 792.0;  % Density of Methanol [mg/ml]
C_Cin = 10.34; % 13C Vanilin stock solution concentration [uM]

%% Section 2: Read file excel
% The file CSV and Excel are opened

% Select CSV file produced by LC-MS
f = msgbox('Choose the excel file produced by the LC-MS.');
file_CSV = uigetfile('*.xls;*.xlsx'); % Open a dialog box to select ...
    the file
delete(f); % Close the window

if ¬file_CSV == 0 % If the file file_CSV exists do...
    % Select the Excel file
    f = msgbox('Choose your excel file, normally the file "vials"');
    file_Excel = uigetfile('*.xls;*.xlsx'); % Open a dialog box to ...
        select the file
    delete(f); % Close the window
    if file_Excel == 0 % If the file file_Excel doesn't exist, prompt ...
        an error
         f = errordlg('You did not select your excel file. Start again ...
             the program and choose a file when asked.');
    end
else % Else if the file file_CSV doesn't exist, prompt an error
    f = errordlg('You did not select the LS-MS excel file. Start ...
        again the program and choose a file when asked.');
end

% Read files CSV and Excel
[¬, ¬, Data_file_CSV]  = xlsread(file_CSV);
[¬, ¬, Data_file_Excel]  = xlsread(file_Excel);

% Convert 'NF' to 0 to ease further calculation
Area = cellfun(@(x)isequal(x,'NF'),Data_file_CSV);
[row_NF, col_NF] = find(Area); % 'Area' is situated at the row "Row" ...
    and at the column "AC"
Data_file_CSV(row_NF, col_NF) = {0};

%% Section 3: Find the location of the required data
% We want to retrive the samples filenames, the type of the sample (blank
% or measure), the measured area of vanilin and Internal STD.
% FNC = FileName_Column, STC = Sample_Type_Column, AC = Area_Column, ...
    ISTDC =
% Internal_Standard_Column

% This function finds the position of the cell containing "Filename"
Filenames = cellfun(@(x)isequal(x,'Filename'),Data_file_CSV);
```

```
[Row, FNC] = find(Filenames); % 'Filename' is situated at the row ...
    "Row" and at the column "FNC"

% This function finds the position of the cell containing "Sample Type"
Sample_Type = cellfun(@(x)isequal(x,'Sample Type'),Data_file_CSV);
[¬, STC] = find(Sample_Type); % 'Sample Type' is situated at the row ...
    "Row" and at the column "STC"

% This function finds the position of the column containing "Area"
Area = cellfun(@(x)isequal(x,'Area'),Data_file_CSV);
[¬, AC] = find(Area); % 'Area' is situated at the row "Row" and at ...
    the column "AC"
AC = AC(end); % "Area" appears in multiple locations but we only need ...
    the last one
% TODO: Find a better way to find the area

% This function finds the position of the column containing the area
ISTD_Area = cellfun(@(x)isequal(x,'ISTD Area'),Data_file_CSV);
[¬, ISTDC] = find(ISTD_Area); % 'ISTD Area' is situated at the row ...
    "Row" and at the column "ISTDC"

%% Section 4: Creation of the pre-treatment cell structure
% In the raw data in the CSV file, the Sample Type can either be "Blank
% Sample" or "Unknown Sample". We only need the samples of type "Unknown
% Sample"

% I want to retrieve the number of rows 'r' in the CSV file to loop ...
    through it
[r, ¬] = size(Data_file_CSV);

% Create the first row of the cell structure
Data_pretreat_CSV = {'Filename', 'Sample Type', 'Area', 'ISTD Area'};

step = 2;
for i = 1:r % loop through all the rows and if
    if ischar(Data_file_CSV{Row+i, STC}) % the content is of type ...
        char and
        if convertCharsToStrings(Data_file_CSV{Row+i, STC}(1:7)) == ...
            "Unknown" % if the content contains Unknown
            % Save the data we are interested for in the cell structure
            Data_pretreat_CSV{step,1} = Data_file_CSV{Row+i, FNC};
            Data_pretreat_CSV{step,2} = Data_file_CSV{Row+i, STC};
            Data_pretreat_CSV{step,3} = Data_file_CSV{Row+i, AC};
            Data_pretreat_CSV{step,4} = Data_file_CSV{Row+i, ISTDC};
            step = step+1; % Increase the step
        end
    else
        break;
    end
end

%% Section 5: Create new cell structure containing also the data from ...
    the Excel file

[r, ¬] = size(Data_file_Excel);

% Create the first row of the cell structure
Data_pretreat_2files = {'Filename', 'Area', 'ISTD Area', 'Exp. [mg]',...
```

```
    'MeOH - C13 + Exp', 'Lignin'};

for i = 2:step-1 % loop through the rows skipping the first one of titles
    % SNLong is the Filename created by the LC-MS
    SNLong = Data_pretreat_CSV{i,1};
    for j = 2:r
        % SNShort is the Filename in the file Excel in the format NNN_L_P
        % Where N = 0-9, L = A/B/C/D/E, P = W or T
        SNShort = Data_file_Excel{j,1}(6:end);
        if contains(SNLong, SNShort) % If the Original Filename ...
            contains the short piece of name
            Data_pretreat_2files{i,1} = SNShort; % Add the short name
            Data_pretreat_2files{i,2} = Data_pretreat_CSV{i,3}; % Add ...
                the Area
            Data_pretreat_2files{i,3} = Data_pretreat_CSV{i,4}; % Add ...
                the ISTD Area
            Data_pretreat_2files{i,4} = Data_file_Excel{j,2}; % Add ...
                mass of experiemnt
            Data_pretreat_2files{i,5} = Data_file_Excel{j,3}; % Add ...
                mass of methanol
            Data_pretreat_2files{i,6} = Data_file_Excel{j,4}; % Add ...
                mass of lignin
        end
    end
end

%% Section 6: Calculations
% The aim of this section is to calculate the mass and yield of vanillin
% produced at the end of the polymerization

% Add new columns to the cell structure to fill with the results
Data_pretreat_2files{1,7} = {'C13 [uM]'};
Data_pretreat_2files{1,8} = {'Dilution Factor'};
Data_pretreat_2files{1,9} = {'Vanilin [uM]'};
Data_pretreat_2files{1,10} = {'Vanilin [mg]'};
Data_pretreat_2files{1,11} = {'yield [%]'};


for i = 2:step-1 % loop through the rows skipping the first one of titles
    m_MeOH_C13 = (Data_pretreat_2files{i,5} - Data_pretreat_2files ...
        {i,4}); %[mg]

    % If the name of the sample contains W use density water otherwise
    % the density of toluene
    if Data_pretreat_2files{i,1}(7) == 'W'
        m_exp = Data_pretreat_2files{i,4};% [mg]
        V_exp = m_exp/rhoW; %[mL]
    else
        m_exp = Data_pretreat_2files{i,4};% [mg]
        V_exp = m_exp/rhoT; %[mL]
    end
    V_MeOH_C13 = m_MeOH_C13/rhoM; %[mL]
    Vtot = V_exp+V_MeOH_C13;
    C_Cfin = (C_Cin*V_MeOH_C13)/Vtot; %[uM]
    f_dil = V_exp/Vtot;

    % Add the calculated data to the cell structure
    Data_pretreat_2files{i,7} = C_Cfin;
```

```
    Data_pretreat_2files{i,8} = f_dil;
end

%% Section 7: Data treatment
% Create the cell structure DataFinal and a temporary cell
% structure DataTemp. In this section the data are separated per sample
% number (e.g. 001) and type of phase (water or toluene) and saved ...
    into the
% temporary cell structure, elaborated and permanently saved in the ...
    DataFinal cell
% structure

% Create empty cell DataTemp
DataTemp = zeros(1, step-2);
DataFinal = {'Sample', 'Area', 'ISTD Area', 'Exp. [mg]', 'MeOH - C13 ...
    + Exp', ...
    'Lignin', 'Dilution Factor', 'C_ISTD', '<C_ISTD>', '||C_ISTD||', ...
        '||A_XX||', ...
    '<||A_XX||>', 'Cv'};
DataFinal2 = {'Sample', 'Area', 'ISTD Area', 'Exp. [mg]', 'MeOH - C13 ...
    + Exp', ...
    'Lignin', 'Dilution Factor', 'C_ISTD'};

for i = 2:step-1 % loop through the rows skipping the first one of titles
    % Convert the sample name from char to string and save it in the
    % DataTemp cell structure
    DataTemp(i-1) = str2double(Data_pretreat_2files{i,1}(1:3));
end

% Determine the number of experiments (e.g. 001, 002 , ...)
Num_Exp = length(unique(DataTemp));
step_2 = 1;
step_3 = 2;

for i = 1:Num_Exp % from 1 to number of experiments
    % create a char for the number of experiment (001, 002, ...)

    Current_Num = unique(DataTemp);
    Current_Num = Current_Num(i);

    % Here we concatenate two strings, the zeros before the number ...
        and the
    % current number itself. If the number is smaller than 10, we ...
        need two
    % zeros, if it is smaller than 100, we need 1 zero and otherwise we
    % need 0 zeros.
    if Current_Num < 10
        char_cat = convertStringsToChars(strcat('00', ...
            num2str(Current_Num)));
    elseif Current_Num < 100
        char_cat = convertStringsToChars(strcat('0', ...
            num2str(Current_Num)));
    else
        char_cat = convertStringsToChars(num2str(Current_Num));
    end

    % Find the index of the rows where the sample name (e.g. 001 for ...
        first loop)
```

```
% appears. Indexes represent the index of the rows where the sample
% name appears. It can be a double or an array if multiple ...
    experiments.
Indexes = ...
    find(cellfun('length',regexp(Data_pretreat_2files(2:end,1), ...
    char_cat)) == 1)+1;

% Create empty cell structures for toluene and water results and ...
    another temp
% cell structure to save temporarelz the data
DataTemp2 = cell(1,1);
DataT = {};
DataW = {};

% We want to target only the cells at the rows 'Indexes' to separate
% the results
for j = 1:length(Indexes) % loop through the number of rows ...
    containing char_cat
    DataTemp2{j,1} = Data_pretreat_2files{Indexes(j),1};
    DataTemp2{j,2} = Data_pretreat_2files{Indexes(j),2};
    DataTemp2{j,3} = Data_pretreat_2files{Indexes(j),3};
    DataTemp2{j,4} = Data_pretreat_2files{Indexes(j),4};
    DataTemp2{j,5} = Data_pretreat_2files{Indexes(j),5};
    DataTemp2{j,6} = Data_pretreat_2files{Indexes(j),6};
    DataTemp2{j,7} = Data_pretreat_2files{Indexes(j),8};
    DataTemp2{j,8} = Data_pretreat_2files{Indexes(j),7};
end

% Find the index of the rows where respectively 'T' or 'W' appear in
% the DataTemp2 cell structure
IndexesT = find(cellfun('length',regexp(DataTemp2(1:end,1),'T')) ...
    == 1);
IndexesW = find(cellfun('length',regexp(DataTemp2(1:end,1),'W')) ...
    == 1);

% Save the toluene results in the toluene structure. Refer to ...
    Section 8
k = 1; % At every loop this has to go back to 1
DataT = SaveDataT(DataT, IndexesT, DataTemp2);
sizeT = size(DataT);

% Save the water results in the water structure. Refer to Section 8
z=1; % At every loop this has to go back to 1
DataW = SaveDataW(DataW, IndexesW, DataTemp2);
sizeW = size(DataW);

n = 1;
% check if the results for that sample (e.g. 001) exist both for ...
    water
% and toluene
if ¬isempty(DataW) && ¬isempty(DataT)
    n = 2;
end

l = 1;
if n == 1 % if exist only for one phase, add the results of the ...
    only phase existing
    if ¬isempty(DataW)
```

```
            % Save the data from the structure DataT to the final ...
               structure
            % DataFinal. Refer to the Section 8.
            [DataFinal, step_2] = InsertDataW(DataFinal, sizeW, ...
               step_2, DataW);

            %Calculate the <C_ISTD> as the mean of C_ISTD
            DataFinal{step_2, 9} = mean([DataW{:,8}]);

            %Calculate the normalized ||C_ISTD||. Take the value between
            %step (actual row) and the two previous values (one ...
               previous if
            %duplicated and not triplicated) step_2-sizeW(1)+1.
            %Using cellfun, divide each of these three values by the
            %average <C_ISTD>
            DataFinal(step_2-sizeW(1)+1:step_2, 10) = cellfun(@(x) ...
                x/DataFinal{step_2,9},DataFinal(step_2-sizeW(1) ...
                   +1:step_2, 8),'un',0);

            %Calculate normalized Area ||A_XX|| as the area times the
            %normalized C_ISTD divided by the area of ISTD
            for iii = step_2-sizeW(1)+1:step_2
                DataFinal{iii,11} = ...
                   DataFinal{iii,2}*DataFinal{iii,10}/DataFinal{iii,3};
            end

            %Calculate the <||A_XX||> as the average of the three values
            DataFinal{step_2, 12} = ...
               mean([DataFinal{step_2-sizeW(1)+1:step_2, 11}]);

            %Calculate Cv
            DataFinal{step_2, 13} = ...
               std([DataFinal{step_2-sizeW(1)+1:step_2, ...
               11}])/DataFinal{step_2, 12}*100;

        else % Only the values for Toluene
            % Save the data from the structure DataT to the final ...
               structure
            % DataFinal. Refer to Section 8.
            [DataFinal, step_2] = InsertDataT(DataFinal, sizeT, ...
               step_2, DataT);

            %Calculate the <C_ISTD> as the mean of C_ISTD
            DataFinal{step_2, 9} = mean([DataT{:,8}]);

            %Calculate the normalized ||C_ISTD||. Take the value between
            %step (actual row) and the two previous values (one ...
               previous if
            %duplicated and not triplicated) step_2-sizeT(1)+1.
            %Using cellfun, divide each of these three values by the
            %average <C_ISTD>
            DataFinal(step_2 - sizeT(1)+1:step_2, 10) = cellfun(@(x)...
                x/DataFinal{step_2,9},DataFinal(step_2-sizeT(1) ...
                   +1:step_2, 8),'un',0);

            %Calculate normalized Area ||A_XX|| as the area times the
            %normalized C_ISTD divided by the area of ISTD
            for kkk = step_2-sizeT(1)+1:step_2
```

```
                DataFinal{kkk,11} = ...
                   DataFinal{kkk,2}*DataFinal{kkk,10}/DataFinal{kkk,3};
            end

            %Calculate the <||A_XX||> as the average of the three values
            DataFinal{step_2, 12} = ...
               mean([DataFinal{step_2-sizeT(1)+1:step_2, 11}]);

            %Calculate Cv
            DataFinal{step_2, 13} = ...
               std([DataFinal{step_2-sizeT(1)+1:step_2, ...
               11}])/DataFinal{step_2, 12}*100;
        end

    else % otherwise, add both

        for JJJ = 1:(sizeT(1)+sizeW(1))/2
            DataFinal2{step_3, 1} = DataT{JJJ,1};
            DataFinal2{step_3, 2} = DataT{JJJ,2};
            DataFinal2{step_3, 3} = DataT{JJJ,3};
            DataFinal2{step_3, 4} = DataT{JJJ,4};
            DataFinal2{step_3, 5} = DataT{JJJ,5};
            DataFinal2{step_3, 6} = DataT{JJJ,6};
            DataFinal2{step_3, 7} = DataT{JJJ,7};
            DataFinal2{step_3, 8} = DataT{JJJ,8};

            DataFinal2{step_3+1, 1} = DataW{JJJ,1};
            DataFinal2{step_3+1, 2} = DataW{JJJ,2};
            DataFinal2{step_3+1, 3} = DataW{JJJ,3};
            DataFinal2{step_3+1, 4} = DataW{JJJ,4};
            DataFinal2{step_3+1, 5} = DataW{JJJ,5};
            DataFinal2{step_3+1, 6} = DataW{JJJ,6};
            DataFinal2{step_3+1, 7} = DataW{JJJ,7};
            DataFinal2{step_3+1, 8} = DataW{JJJ,8};
            step_3 = step_3 + 2;
        end

        % Save the data from the structure DataT to the final structure
        % DataFinal. Refer to Section 8.
        [DataFinal, step_2] = InsertDataW(DataFinal, sizeW, step_2, ...
           DataW);

        %Calculate the <C_ISTD> as the mean of C_ISTD
        DataFinal{step_2, 9} = mean([DataW{:,8}]);

        %Calculate the normalized ||C_ISTD||. Take the value between
        %step (actual row) and the two previous values (one previous if
        %duplicated and not triplicated) step_2-sizeW(1)+1.
        %Using cellfun, divide each of these three values by the
        %average <C_ISTD>
        DataFinal(step_2-sizeW(1)+1:step_2, 10) = cellfun(@(x) ...
            x/DataFinal{step_2,9},DataFinal(step_2-sizeW(1)+1:step_2, ...
               8),'un',0);

        %Calculate normalized Area ||A_XX|| as the area times the
        %normalized C_ISTD divided by the area of ISTD
        for iii = step_2-sizeW(1)+1:step_2
            DataFinal{iii,11} = ...
```

```
               DataFinal{iii,2}*DataFinal{iii,10}/DataFinal{iii,3};
        end

        %Calculate the  <||A_XX||> as the average of the three values
        DataFinal{step_2, 12} = ...
           mean([DataFinal{step_2-sizeW(1)+1:step_2, 11}]);

        %Calculate Cv
        DataFinal{step_2, 13} = ...
           std([DataFinal{step_2-sizeW(1)+1:step_2, ...
           11}])/DataFinal{step_2, 12}*100;

        % ||||||| Here begins for the toluene values |||||||

        % Save the data from the structure DataT to the final structure
        % DataFinal. Refer to the Section 8.
        [DataFinal, step_2] = InsertDataT(DataFinal, sizeT, step_2, ...
           DataT);

        %Calculate the <C_ISTD> as the mean of C_ISTD
        DataFinal{step_2, 9} = mean([DataT{:,8}]);

        %Calculate the normalized ||C_ISTD||. Take the value between
        %step (actual row) and the two previous values (one previous if
        %duplicated and not triplicated) step_2-sizeT(1)+1.
        %Using cellfun, divide each of these three values by the
        %average <C_ISTD>
        DataFinal(step_2 - sizeT(1)+1:step_2, 10) = cellfun(@(x)...
            x/DataFinal{step_2,9},DataFinal(step_2-sizeT(1)+1:step_2, ...
               8),'un',0);

        %Calculate normalized Area ||A_XX|| as the area times the
        %normalized C_ISTD divided by the area of ISTD
        for kkk = step_2-sizeT(1)+1:step_2
            DataFinal{kkk,11} = ...
               DataFinal{kkk,2}*DataFinal{kkk,10}/DataFinal{kkk,3};
        end

        %Calculate the  <||A_XX||> as the average of the three values
        DataFinal{step_2, 12} = ...
           mean([DataFinal{step_2-sizeT(1)+1:step_2, 11}]);

        %Calculate Cv
        DataFinal{step_2, 13} = ...
           std([DataFinal{step_2-sizeT(1)+1:step_2, ...
           11}])/DataFinal{step_2, 12}*100;

    end
end

%% Section 8: Helper functions
function DataT = SaveDataT(DataT, IndexesT, DataTemp2)
% This functions takes as input the strcutres DataT (toluene) and ...
   DataTemp2 and
% update the DataT by adding the values od DataTemp2 at IndexesT. It
% returns the updated version of DataT.
for k = 1:length(IndexesT)
    DataT{k,1} = DataTemp2{IndexesT(k),1};
```

```
    DataT{k,2} = DataTemp2{IndexesT(k),2};
    DataT{k,3} = DataTemp2{IndexesT(k),3};
    DataT{k,4} = DataTemp2{IndexesT(k),4};
    DataT{k,5} = DataTemp2{IndexesT(k),5};
    DataT{k,6} = DataTemp2{IndexesT(k),6};
    DataT{k,7} = DataTemp2{IndexesT(k),7};
    DataT{k,8} = DataTemp2{IndexesT(k),8};
end
end

function DataW = SaveDataW(DataW, IndexesW, DataTemp2)
% This functions takes as input the strcutres DataW (water) and ...
    DataTemp2 and
% update the DataW by adding the values od DataTemp2 at IndexesW. It
% returns the updated version of DataW.
for z = 1:length(IndexesW)
    DataW{z,1} = DataTemp2{IndexesW(z),1};
    DataW{z,2} = DataTemp2{IndexesW(z),2};
    DataW{z,3} = DataTemp2{IndexesW(z),3};
    DataW{z,4} = DataTemp2{IndexesW(z),4};
    DataW{z,5} = DataTemp2{IndexesW(z),5};
    DataW{z,6} = DataTemp2{IndexesW(z),6};
    DataW{z,7} = DataTemp2{IndexesW(z),7};
    DataW{z,8} = DataTemp2{IndexesW(z),8};
end
end

function [DataFinal, step_2] = InsertDataT(DataFinal, sizeT, step_2, ...
    DataT)
% This functions takes as input the structres DataFinal and DataT ...
    (toluene)
% the size (sizeT) of the DataT structure, the actual value of step_2 ...
    that changes
% during the loop. It loops through the number of rows available in ...
    DataT,
% known as sizeT(1) which are normally 3 since the experiments are
% triplicated (but it could happens to have less) and update the ...
    structure
% DataFinal. It returns the updated structure DataFinal and the new ...
    step_2
% value.
for kk = 1:sizeT(1)
    step_2 = step_2 + 1;
    DataFinal{step_2, 1} = DataT{kk,1};
    DataFinal{step_2, 2} = DataT{kk,2};
    DataFinal{step_2, 3} = DataT{kk,3};
    DataFinal{step_2, 4} = DataT{kk,4};
    DataFinal{step_2, 5} = DataT{kk,5};
    DataFinal{step_2, 6} = DataT{kk,6};
    DataFinal{step_2, 7} = DataT{kk,7};
    DataFinal{step_2, 8} = DataT{kk,8};
end
end

% Save the data from the DataT to the final structure
function [DataFinal, step_2] = InsertDataW(DataFinal, sizeW, step_2, ...
    DataW)
% This functions takes as input the structres DataFinal and DataW (water)
```

```
% the size (sizeW) of the DataW structure, the actual value of step_2 ...
    that changes
% during the loop. It loops through the number of rows available in ...
    DataT,
% known as sizeT(1) which are normally 3 since the experiments are
% triplicated (but it could happens to have less) and update the ...
    structure
% DataFinal. It returns the updated structure DataFinal and the new ...
    step_2
% value.
for ii = 1:sizeW(1)
     step_2 = step_2 + 1;
     DataFinal{step_2, 1} = DataW{ii,1};
     DataFinal{step_2, 2} = DataW{ii,2};
     DataFinal{step_2, 3} = DataW{ii,3};
     DataFinal{step_2, 4} = DataW{ii,4};
     DataFinal{step_2, 5} = DataW{ii,5};
     DataFinal{step_2, 6} = DataW{ii,6};
     DataFinal{step_2, 7} = DataW{ii,7};
     DataFinal{step_2, 8} = DataW{ii,8};
end
end
```

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