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Recovery of Valuable Chemicals from Agricultural Waste Through Pyrolysis

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Abstract

Agricultural crop residues are a source of inexpensive biomass to convert into bioproducts. The recovery of valuable chemicals from plant waste would partly solve the disposal issue and offer a more environmentally friendly alternative to synthetic chemical production. One approach to separate and concentrate valuable chemicals from biomass is pyrolysis using a batch reactor process. A mechanically fluidized reactor (MFR) was developed to pyrolyze biomass from ambient to temperatures near 600 °C, forming gases that are then condensed in an ice-chilled condenser to form a bio-oil. The bio-oil produced by the MFR can be separated within temperature ranges, termed one-dimensional or 1-D pyrolysis. Further separation of bio-oil can be achieved by two condensers, one set at a high and one at a low temperature, to isolate the gases by boiling point, termed two-dimensional or 2-D pyrolysis. Char is a by-product of pyrolysis and can be converted into a value-added product, activated carbon, by heating using a jiggle bed reactor (JBR). The biomass investigated, tobacco leaf, tomato plant, spent coffee ground, and hydrolysis and organosolv lignin, were chosen based on availability and the valuable products previously identified in the bio-oil. The thesis objectives were: 1) optimize the 1-D and 2-D MFR for chemical recovery; 2) to isolate bio-oil fractions containing pesticide and antioxidant activity with the 2-D MFR and 3) to compare the activated carbon produced by the JBR from char. After 2-D MFR pyrolysis, a total nicotine recovery of 90% from tobacco bio-oil compared to solvent extraction was obtained when the nicotine concentration was 20%. Both the tobacco and tomato bio-oils could be separated through the 2-D MFR to isolate fractions with high insecticide activity, and the antioxidant concentration in the tomato and organosolv lignin bio-oil was 97 and 91%, respectively. The tomato char produced the activated carbon with the highest adsorption capacity, comparable to commercial coconut shell. In summary, valuable products including pesticides, antioxidants and activated carbon can be recovered from crop waste by MFR pyrolysis demonstrating a successful example of a biorefinery, a sustainable process for converting biomass into a range of bio-based products.
Keywords

Pesticides, Antioxidant, Activated carbon, Biomass, Pyrolysis, Mechanically fluidized reactor, Biorefinery.
Co-Authorship Statement

Chapter 2: Optimizing pyrolysis reactor operating conditions to increase nicotine recovery from tobacco leaves

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Chapter 3: A two-dimensional pyrolysis process to concentrate nicotine during tobacco leaf bio-oil production

This chapter will be submitted to the journal, Bioresource Technology.

Authors: Hossain M.M., Scott I.M., Berruti F., Briens C.

The experimental work was conducted by Mohammad Hossain under the supervision of Dr. Cedric Briens, Dr. Franco Berruti and Dr. Ian Scott. Writing and data analysis were conducted by Mohammad Hossain. It was reviewed and revised by Dr. Cedric Briens, Dr. Franco Berruti and Dr. Ian Scott.

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This chapter will be submitted to the journal, Separation and Purification Technology.

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**Chapter 6: Comparison of activated carbon from biochar produced by pyrolysis of tobacco leaf and tomato plant residue**

This chapter will be submitted to the journal, Fuel Processing Technology.

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**Chapter 7: Application of 1-D and 2-D MFR reactor technology: Hydrolysis and organosolv lignins**

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Dedication

This thesis is dedicated to my Mother and Father
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Abbreviations

1-D: one dimensional
2-D: two dimensional
ANOVA: analysis of variance
CL: cabbage looper
CM: *Clavibacter michiganensis* subsp. *michiganensis*
CPB: Colorado potato beetle
d.f.: degree of freedom
ESP: electrostatic precipitator
F.L.: fiducial limit
F: F- statistic
GC-FID: gas chromatography-flame ionization detector
GC-MS: gas chromatography-mass spectrometry
GLM: generalized linear model
HPLC: high performance liquid chromatography
HSD: honest significant difference
JBR: jiggled bed reactor
kDa: kilodalton
LC\textsubscript{50}: an LC\textsubscript{50} is the lethal concentration required to kill 50\% of the population
MB: methylene blue
MFR: mechanically fluidized reactor
P: in statistics, the p-value is a function of the observed sample results that is used for testing a statistical hypothesis
PID: proportional integral derivative
PROC: procedure
psi: pounds per square inch
PU: *Pythium ultimum*
r.c.f.: relative centrifugal force
r.p.m.: rotations per minute
RH: relative humidity
RO: reverse osmosis
RS: *Rhizoctonia solani*
S.E.: standard error
SAS: statistical analysis system
SS: *Sclerotinia sclerotiorum*
TSSM: two-spotted spider mite
UV-Vis: ultraviolet-visible spectroscopy
XC: *Xanthomonas campestris* pv. *vesicatoria*
Chapter 1

1 Introduction

1.1 Biomass pyrolysis

Biomass has the potential to provide renewable products for the future, either in the form of gas (e.g., CH₄), liquid (e.g., bio-oil) or solid (e.g., char). Thermochemical conversion process is one of the main routes to provide these products since this process provides a wide range of energy, fuel and chemical options [Bridgwater 2006]. Pyrolysis is a thermochemical conversion of organic material at high temperature in the absence of air and can be a continuous or batch process. The MFR can be a continuous process with constant biomass feeding and char removal. In this work, the MFR is used for batch pyrolysis: because the biomass feedstock is heated progressively, pyrolysis is classified as slow [Bridgwater 2006]. The bio-oil is collected in a condenser after the set temperature pyrolysis of biomass. Note that Fig. 1.1 shows a laboratory implementation where the char produced in each run is allowed to build up in the bed. Adjustable heating rates allow the MFR bed temperature to be raised from ambient to the desired temperature. The MFR bio-oil can be fractionated by using different reactor temperature cuts, and no fluidizing gas (e.g., nitrogen gas) or bed material (e.g., sand) is required [Hossain 2011]. The MFR has a stirrer with variable r.p.m. which provides the uniform heat distribution in the biomass. As with the fluidized reactor, three types of products are formed when the biomass is pyrolyzed: gas, liquid and char.

The two main pyrolysis reactors used in industry are 1) the auger reactor and 2) the fluidized bed reactor. The auger reactor is also a slow pyrolysis reactor. The biomass can be pyrolyzed continuously to produce bio-oil in a single screw auger reactor by using sand as an inert bed material [Veses et al. 2015]. When the biomass is pyrolyzed in an auger reactor in a continuous process [Ravindran et al. 2015] it is difficult to isolate the compounds based on their boiling points. The auger reactor does not need any carrier gas and can work at low pyrolysis temperature [Mohan et al. 2006]. Fluidized bed reactor
uses nitrogen gas and sand as the fluidizing gas and the bed material, respectively and pyrolysis occurs at a fixed reactor temperature. Traditional fast fluidized bed pyrolysis is normally a continuous process; some researchers have operated a fluidized bed with continuous biomass feeding and no char removal, for short runs to minimize char accumulation [Fig. 1.1 adapted from Cáceres et al. 2015].

Fig. 1.1 Schematic process flow diagram of fluidized bed reactor [adapted from Cáceres et al. 2015].

1.2 Agricultural crop waste: a source of biomass and value added products

Agricultural crop residues protect soil erosion and increase crop nitrogen (N) supply [Li et al. 2016], but these objectives can be met with only a fraction of the residues: the remainder of the residue is considered as agricultural waste. Globally, the renewable energy contributes 19% of the total energy consumption. Out of which, 9.3% is from
traditional biomass [Edrisi and Abhilash 2016]. This thesis focuses on developing applications for tobacco leaf, tomato plant residue, spent coffee ground and lignin.

World tobacco leaf production was about 500 million tons in 2006, and more than 20% of the leaf resources are discarded as waste and pollutes the environment [Wang et al. 2008, Hu et al. 2015]. The discarded tobacco leaves are economically valuable because of the bioactive compounds, including nicotine [Wang et al. 2008].

Canada produced 540 ha of greenhouse tomato with a total production was 268,502 tons in 2011 [Statistics Canada 2011]. The tomato plant material becomes a waste biomass and can also be extracted to recover pesticidal compounds [Cáceres et al. 2015]. The additional recovery of char for the conversion to activated carbon would provide additional value for both tobacco [Kilic et al. 2011] and tomato [Dasgupta et al. 2015] biomasses.

Coffee grounds are produced in large quantities globally and an application for this material is an alternative to disposal in landfill. The global coffee production was 143.25 million 60 kg bags in 2014 [International Coffee Organization 2015]. Our research group previously produced pesticidal compounds through pyrolysis [Booker et al. 2010, Bedmutha et al. 2011, Hossain 2011]. The bio-oil from spent coffee grounds produces pesticidal compounds [Bedmutha et al. 2011], but, solvent extraction was required to isolate the pesticidal compounds. The problem with solvent extraction is that it is time consuming, expensive and not environmentally friendly.

Hydrolysis and organosolv lignin produced as a by-product in bioethanol industries [Kuglarz et al. 2016, Mesa et al. 2016] can also be a source, for example, of pesticidal compounds [Hossain et al. 2013].

Several potential applications are investigated in this thesis. Pyrolysis is used to produce nicotine, pesticides, antioxidants and activated carbon.
1.2.1 Nicotine

Many plant-derived compounds are considered valuable chemicals. Although cigarette smoking is one of the most important risk factors that develop various diseases [Tsunoda et al. 2016], the alkaloid, nicotine, commonly found in solanaceous plants, has been used both as an insecticide and drug, including medications for several neuropsychiatric disorders. Nicotine is the main alkaloid in tobacco (Nicotiana tabacum), comprising approximately 95% of the total alkaloid content in tobacco leaves [Schmeltz and Hoffmann 1977]. The level of nicotine in different tobacco cultivars (Nicotiana tabacum) ranges from 0.3 to 3% (Table 1.1 data from Tassew and Chandravanshi 2015). Nicotine is recommended as a therapeutic agent for people with mild cognitive impairment [Newhouse et al. 2012; Heishman et al. 2010] and can be used to prevent tumor formation [Jin et al. 2013]. Nicotine and its metabolites [Liu et al. 2015] also reduce the symptoms of experimental autoimmune encephalomyelitis [Gao et al. 2015], Parkinson’s disease [Quik et al. 2008], Alzheimer’s disease [Levin and Rezvani 2000] and is widely used in alternative therapies for smoking cessation [Hukkanen et al. 2005].

Table 1.1 Comparison of nicotine level (%) of Ethiopian tobacco leaves based on their species.

<table>
<thead>
<tr>
<th>Type of species of tobacco</th>
<th>Average nicotine level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virginia (Billate)</td>
<td>3.26 ± 0.16</td>
</tr>
<tr>
<td>Virginia (Shewa Robit)</td>
<td>2.20 ± 0.07</td>
</tr>
<tr>
<td>Burley (Awassa)</td>
<td>0.650 ± 0.02</td>
</tr>
<tr>
<td>Oriental (Awassa)</td>
<td>ND (≤ 0.050)</td>
</tr>
<tr>
<td>Gaya (Wollayita)</td>
<td>1.11 ± 0.05</td>
</tr>
</tbody>
</table>

ND: not detected. It was roughly equal to detection limit

1.2.2 Biopesticides

Petroleum oil is the main source of synthetic pesticides and one way to reduce the dependency on oil is to use alternative pest management strategies. The synthetic pesticide, Admire® 240 FS (active ingredient: imidacloprid) is 39000-fold more toxic
than the organosolv lignin ESP fraction in terms of LC$_{50}$ (mg/mL) [Hossain et al. 2013]. But the use of synthetic pesticides is declining due to government legislation and the problems associated with resistance in pest populations [Chandler et al. 2011]. Biopesticides, for example, are very effective for agricultural crop pest control without creating serious health or environmental problems [Leng et al. 2011]. Bio-oil biopesticides could have negative effects on the environment if there are some toxic non-target compounds that occur, for instance polycyclic aromatic hydrocarbons (PAH) [Hossain et al. 2013]. In previous studies it was shown that pyrolysis of biomass could produce bio-oils that contain compounds with biopesticide activity. Tobacco leaf bio-oil was produced using a fluidized bed reactor. The bioassays with the Colorado potato beetle (CPB) *Leptinotarsa decemlineata* L. (Coleoptera: Chrysomelidae) and three microorganisms (*Streptomyces scabies*, *Clavibacter michiganensis* and *Pythium ultimum*) showed these pest organisms were strongly affected by the bio-oil produced at different pyrolysis temperatures [Booker et al. 2010]. Similarly, when dried coffee ground bio-oil was produced through pyrolysis using a fluidized bed reactor the bio-oil had strong insecticidal activity against the CPB and bactericidal property against *S. scabies* and *C. michiganensis* [Bedmutha et al. 2011]. A study of bio-oil from the pyrolysis of greenhouse tomato plant residues identified phytol, neophytadiene and several fatty acids in the active non-polar bio-oil fraction, but only with combinations of these compounds was the greatest insecticidal activity observed [Cáceres et al. 2015]. In contrast, the insecticidal and feeding repellent activity of canola (*Brassica napus*) straw and mustard (*B. carinata* and *B. juncea*) straw bio-oil aqueous fractions was equally as effective as the non-polar fractions. The benefit of these findings was that the aqueous fractions of the bio-oil did not contain undesirable phenol compounds observed in other non-polar fractions. It was determined that hexadecanoic and octadecanoic fatty acids in the active bio-oil fraction partly contributed to the insecticidal activity of the bio-oil [Suqi et al. 2014]. When organosolv lignin, cellulose and hemicellulose, individually, as well as in mixture, were pyrolyzed in a fast fluidized bed reactor the combination of two biomass components exhibited synergism in the insecticidal activity bioassay [Hossain et al. 2013]. In contrast, the organosolv lignin-cellulose-hemicellulose bio-oil combination exhibited an antagonistic effect.
1.2.3 Antioxidants

An antioxidant is a compound that inhibits the oxidation of other compounds and many of the plant-derived antioxidant compounds have an essential role in the human diet [Balasundram et al. 2006]. Biomass conversion through thermochemical means, principally pyrolysis, can yield products with antioxidant properties. Several examples include, pyroligneous acid obtained from *Schisandra chinensis* [Ma et al. 2014], Japanese red pine (*Pinus densiflora* Siebold and Zucc.) [Patra et al. 2015] and a phenolic extract (PE) isolated from *Salix viminalis* pyrolysis bio-oil [Ilnicka et al. 2014]. Of the pyroligneous acids produced from walnut shell at three different temperature ranges (90-150, 151-310 and 311-550 °C), the acid collected from the high temperature range showed the strongest antioxidant activity [Wei et al. 2010]. These studies indicate the need to explore further: 1) the types of biomass suitable for conversion; 2) the pyrolysis bio-oil components considered as valuable chemicals; 3) the chemicals that can be applied as biopesticides and antioxidants and 4) the reactor and separation technology required to make this process cost-effective.

1.2.4 Activated carbon

Activated carbon is a form of carbon processed to have low-volume pores that increase the surface area available for adsorption. Activated carbon has been used as adsorbent in many applications in which contaminants in low concentration are removed. Numerous precursors have been used for the production of activated carbons including waste tea [Yagmur et al. 2008] and tomato stem [Dasgupta et al. 2015]. There are two processes for the preparation of activated carbon: physical and chemical activation [Yagmur et al. 2008]. Physical activation involves carbonization of a carbonaceous material followed by activation of the resulting char in the presence of mildly oxidizing activating agents such as CO$_2$ or H$_2$O [Colomba 2015]. In the chemical activation, the raw material is impregnated with activating agents such as H$_3$PO$_4$; then the impregnated material is heat-treated at various temperatures [Yagmur et al. 2008]. Activated carbon can also be produced by physical activation from residues such as olive residue and kraft lignin char in a jiggled bed reactor (JBR) [Colomba 2015]. The char activation temperature range
was 800 to 900 °C. The adsorption capacity of the activated carbon was evaluated with methylene blue (MB) in aqueous solutions. The adsorption capacity for the olive residue was approximately 50 mg MB/g activated carbon whereas the adsorption capacity for the kraft lignin was 100 mg MB/g activated carbon. For this study, activated carbon was produced from tobacco leaf, tomato plant, hydrolysis lignin and organosolv lignin through physical activation.

1.3 Rationale

All of the biomass mentioned, greenhouse tomato waste, tobacco leaf, spent coffee grounds, hydrolysis and organosolv lignin are sources of biomass readily available in Canada. Therefore, it is worthwhile to produce valuable chemicals (e.g., biopesticides, antioxidants, etc.) from these biomasses through pyrolysis. In this thesis, my objectives were to produce highly concentrated fractions with high activity from waste biomass through an MFR thus avoiding or minimizing several steps of solvent extraction technique and activated carbon as a by-product from pyrolysis char.

1.4 Objectives

The objectives of the thesis are as follows:

1. Optimizing 1-D MFR reactor operating conditions to increase nicotine recovery from the pyrolysis of tobacco leaves;

2. Develop a two-dimensional pyrolysis process to produce highly concentrated liquids and apply it to the concentration of nicotine during bio-oil production from tobacco leaf;

3. Application of 1-D and 2-D MFR reactor technology for the isolation of insecticidal and anti-microbial properties from pyrolysis bio-oils;

4. Application of 1-D and 2-D MFR reactor technology: Antioxidant activities of tobacco leaf, tomato plant residue and spent coffee bio-oils;
5. Comparison of activated carbon from biochar produced by pyrolysis of tobacco leaf and tomato plant residue;

6. Application of 1-D and 2-D MFR reactor technology: Hydrolysis and organosolv lignin biomass.

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Chapter 2

2 Optimizing pyrolysis reactor operating conditions to increase nicotine recovery from tobacco leaves

Abstract

Biomass conversion to obtain bioenergy and fuels will only be cost effective when additional, value-added products including chemicals can be obtained as part of the process. The objective of this study was to increase nicotine recovery from tobacco leaves (Nicotiana tabacum) through pyrolysis using new technology, a mechanically fluidized reactor (MFR). The MFR is a cylindrical batch reactor, 15 cm in diameter and 25.4 cm in height. A condenser was connected to the reactor and the gas was then passed through a cotton demister and exhausted. The gases from the reactor were condensed using condensers in an ice bath and changed according to the temperature of the fluidized reactor bed. Two variables, tobacco leaf particle size (<1, <0.355 and <0.212 mm) and reactor heating rate (5, 10 and 15 °C/min from ambient to 565 °C), were studied in terms of the effect on bio-oil yield, nicotine concentration and nicotine recovery. The bio-oil was collected in nine different temperature cuts (ambient-200, 200-250, 250-275, 275-300, 300-350, 350-400, 400-450, 450-500 and 500-565 °C) with reactor temperatures held for 30 min at 200, 250, 275 and 500 °C. The bio-oil and biomass extracts were analyzed for nicotine by gas chromatography-flame ionization detector (GC-FID). Nicotine recovery was higher than conventional solvent extraction of biomass when tobacco leaves were pyrolyzed by MFR. Biomass particle sizes did not affect either total bio-oil yield or nicotine recovery, which ranged from 48 to 49% and 104 to 109%, respectively. The second temperature cut produced from <1 mm biomass has the highest concentration of nicotine (6%) when the nicotine recovery is 45%. In contrast, the first temperature cut produced from <1 mm biomass and dried at 50 °C has the highest concentration of nicotine (84%) when the nicotine recovery is 23%. Because of the relatively high boiling point of nicotine (247 °C), the cuts obtained from batch pyrolysis
can be greatly purified by evaporating water and other volatiles. This method of nicotine recovery and purification through pyrolysis can be applied to recover other value-added compounds from other biomasses.

**Keywords:** Tobacco leaf; Nicotine; Pyrolysis; Mechanically fluidized reactor.

### 2.1 Introduction

Fossil fuel reserves become more limited by the day, are increasingly expensive to obtain, and their consumption contributes to climate change [Wang et al. 2009]. In contrast, plant biomass in the form of agricultural and forestry waste has little economic value, is renewable and can be a source of valuable chemicals as well as fuel. By obtaining the valuable chemicals from this waste, the cost of biomass conversion and biofuel production will be subsidized and at the same time minimize environmental pollution. Plant biomass can be converted into valuable chemicals through pyrolysis, the chemical conversion of organic material by heating in the absence of oxygen. Agricultural and forestry waste has many advantages compared with fossil fuel since biomass is abundant, carbon dioxide neutral and sustainable [Zhang et al. 2010].

Nicotine is an alkaloid and it comprises approximately 95% of the total alkaloid content in tobacco leaves (*Nicotiana tabacum*) [Schmeltz and Hoffmann 1977]. Nicotine and its metabolites [Liu et al. 2015] reduce the symptoms of Parkinson’s disease [Quik et al. 2008] and Alzheimer’s disease [Levin and Rezvani 2000]. Tobacco bio-oil was produced through pyrolysis of tobacco leaves using a fluidized bed reactor at different temperature (350, 400, 450, 500, 550 and 600 °C) and residence time (5, 10 and 17 s). The bio-oil yield was the highest and it was 43.4% at the pyrolysis temperature of 500 °C and vapor residence time of 5 s [Booker et al. 2010].

The pesticidal activity of tobacco leaf biomass bio-oil produced by fluidized bed reactor was determined to be associated with many small phenol derivatives. The active fractions produced high mortality of Colorado potato beetle larvae and inhibition of the bacteria *Streptomyces scabies* and *Clavibacter michiganensis* subsp. *michiganensis* and fungi *Pythium ultimum* [Booker et al. 2010]. A potential value-added product recovered from
tobacco bio-oil is nicotine, a proven therapeutic agent for enhancing cognitive function of people with mild cognitive impairment [Newhouse et al. 2012, Levin and Rezvani 2000]. Nicotine also had a preventive and therapeutic effect on tumor formation of dendritic cells, one of the most important antigen presenting cells for inducing antigen-specific cytotoxic T-lymphocyte priming [Jin et al. 2013].

The batch mechanically fluidized reactor (MFR) was developed to achieve some liquid separation during pyrolysis by collecting liquids as the reactor temperature was gradually raised [Briens et al. 2010]. For example, in place of liquid-liquid separation of bio-oil, different temperature cuts during the pyrolysis of lignin by a batch mechanically fluidized reactor (MFR) was able to isolate the highest pesticidal activity in the cut between 250 and 300 °C [Hossain 2011]. Further examination confirmed that the bio-oil yield and the concentration of the active compounds were dependent on pyrolysis temperature [Demirbas 2007], indicating the possibility of developing pyrolysis to ensure that valuable chemicals are preserved and collected while converting the biomass into bio-oil, and minimizing the need for other extraction processes.

The objective of this study was to optimize the MFR pyrolysis operating conditions to maximize the recovery and concentration of nicotine from tobacco leaves in order to improve the efficient separation and isolation of this compound during bio-oil production.

2.2 Materials and methods

2.2.1 Plants

Wild type tobacco plants (*Nicotiana tabacum*) were grown at Agriculture and Agri-Food Canada (AAFC), Delhi, Ontario, Canada. Tobacco leaves were dried in an oven at 60 °C and were ground with a Thomas Model 4 Wiley Mill® (Thomas Scientific, Swedesboro, NJ, USA) to <1, <0.355 and <0.212 mm particle sizes. The proximate and ultimate analyses (Table 2.1) of tobacco have been adapted from Senneca et al. [2007]. Cardoso et al. [2013] confirmed the gross calorific value of tobacco waste (12.28 MJ/kg) generated in a tobacco processing plant.
Table 2.1 Proximate and ultimate analyses of tobacco.

<table>
<thead>
<tr>
<th>Proximate analysis</th>
<th>(wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volatiles</td>
<td>61.9</td>
</tr>
<tr>
<td>Ashes</td>
<td>18.1</td>
</tr>
<tr>
<td>Moisture</td>
<td>4.1</td>
</tr>
<tr>
<td>Fixed carbon</td>
<td>15.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ultimate analysis</th>
<th>(wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>38.0</td>
</tr>
<tr>
<td>H</td>
<td>5.5</td>
</tr>
<tr>
<td>N</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Adapted from Senneca et al. [2007].

2.2.2 Chemicals

High performance liquid chromatography grade dichloromethane (DCM) (99.7%) was purchased from Caledon Laboratories Ltd., Canada. Reagent grade sodium hydroxide (NaOH) (97%) and analytical grade (-)-nicotine (98.7%) were purchased from Sigma-Aldrich, Canada.

2.2.3 Bio-oil production

Ground tobacco leaves were pyrolyzed. The reactor heating rate from ambient to 565 °C was 10 °C/min using the MFR located at the Institute for Chemicals and Fuels from Alternative Resources (ICFAR), Western University, London, Ontario, Canada. No fluid (inert gas or liquid) was used to fluidize the biomass in the reactor; rather a stirrer continuously stirred the biomass to achieve the same mixing quality as a traditional fluidized bed [Lago, 2015]. As a result the stirrer provided uniform heat distribution. Therefore, it is considered as an MFR instead of a fixed bed reactor [Hossain 2011, Cascarosa et al. 2011]. The reactor is cylindrical, 15 cm in diameter and 25.4 cm in height (Fig. 2.1). It has two band heaters controlled by a Watlow’s controller (St. Louis, MO, USA).
The biomass was placed in the reactor before the experiment. It is a batch process and the vapor production from the biomass fluctuates during the run, as the reactor temperature is raised from ambient to 565 °C. The residence time of the produced gases from the biomass was not measured. The gases from the reactor were condensed using stainless steel condensers in an ice bath and the gas was then passed through a cotton demister and exhausted. The condensers were changed according to the temperature of the fluidized bed. Based on the nicotine concentration and recovery in the produced bio-oil nine different reactor temperature cuts (ambient-200, 200-250, 250-275, 275-300, 300-350, 350-400, 400-450, 450-500 and 500-565 °C) were optimized. At the reactor temperatures of 200, 250, 275 and 500 °C the temperature was held for 30 min. No holding time was used for the other temperature cuts (Fig. 2.2). At the end of each reactor temperature range, the condenser with the product oil was switched for a clean one. For instance, when the reactor temperature reached 200 °C the temperature was held for 30 min. The bio-oil contained in the condenser was then removed from the vapor production line and a new condenser was connected in the vapor production line for the next bio-oil cut of 200-250 °C. Two batches of biomass for each particle size were pyrolyzed to produce the bio-oils. The viscosity of the bio-oil varied depending on the reactor temperature ranges.
Fig. 2.1 Schematic for the batch mechanically fluidized reactor.
Fig. 2.2 The reactor temperature varied with time and the bio-oil cuts were collected when the tobacco leaves were pyrolyzed through the 1-D MFR from ambient to 565 °C.

2.2.4 Bio-oil sample preparation for Gas Chromatography-Flame Ionization Detector (GC-FID)

Each cut of 100 µL bio-oil was weighed and was mixed with 4 mL Milli-Q water, 2 mL 10% NaOH and 4 mL DCM. The samples were basified by the addition of NaOH. The use of 10% NaOH, while allowing nicotine extraction from bio-oil, lowers its solubility in the aqueous phase [Jamin et al. 1997] and thus facilitates its passage into DCM. The sample was vortexed for 1 min, sonicated for 5 min, shaken for 10 min and was allowed to settle for 3 h for separation of the water and DCM phases. The DCM layer was passed through a 0.2 µm pore size syringe filter with a nylon membrane and 2 µL was injected into the GC-FID (Hewlett Packard 5890 Series II) and compared with a standard curve for nicotine to obtain the nicotine content in the bio-oil. The GC column was manufactured by Phenomenex (ZB-5HT, 30 m × 0.25 mm and film thickness 0.25 µm) (Torrance, CA, USA). The injector temperature was 250 °C and inlet pressure was 7 psi. The inlet was operated in splitless mode. The oven temperature was initially held for 0.5 min at 50 °C, and then increased at 5 °C/min to 125 °C. Next the temperature was
increased at 2 °C/min to 155 °C and held for 8 min. At the final stage the temperature was increased at 25 °C/min to 260 °C and held for 8 min. The detector temperature was 275 °C. The nicotine extraction and quantification method was modified from Kaldis et al. [2013], with the exception that GC-FID and an external standard were added as described by Docheva et al. [2014]. The identity of the chromatographic peak (Fig. 2.3B) was confirmed by comparison of the retention time of the sample with that of the standard (Fig. 2.3A). The standard was run at 7 concentrations, ranging from 0.0396 to 1.5866 mg/mL (0.0396, 0.0793, 0.1587, 0.2380, 0.3173, 0.5288 and 1.5866 mg/L) such that the diluted sample peaks (bio-oil and biomass extracts) fell between 7 standard calibration points. The correlation coefficient ($R^2$) was 0.99965. The recovery of external standard was 99.6%. Nicotine in the biomass extract as well as for all temperature cuts up to 400 °C was identified by Agilent gas chromatography-mass spectrometry (GC-MS) (GC 7890B and MS 5977A) by comparison of its mass spectra with the Wiley/NIST mass spectral library 2008. Identification of nicotine in biomass as well as in bio-oil was further confirmed by use of standard.

2.2.5 Bio-oil dried by nitrogen gas/at 50 °C

Each cut of 100 µL bio-oil was weighed and was dried by passing a gentle stream of nitrogen gas until the bio-oil reached a constant weight. Again, each cut of 100 µL bio-oil was weighed and was dried at 50 °C on a hot plate until the bio-oil reached a constant weight. Analysis of nicotine in the dried samples followed the methods described in subsection 2.2.4. The objective of this method was to determine if the drying effect by either nitrogen gas or heating to a low temperature such as 50 °C would increase the nicotine concentration and recovery in the bio-oil.

2.2.6 Biomass extracts sample preparation for GC-FID

Dried tobacco leaf, 1 g, was dissolved in 10 mL Milli-Q water, 5 mL 10% NaOH and 10 mL DCM in an amber-glass vial. The mixture was then vortexed for 1 min and sonicated for 10 min. The sample was further shaken for 30 min and was then centrifuged for 15 min at 514 relative centrifugal force (r.c.f.) or 1500 r.p.m. It was then allowed to settle for
3 h for separation of water and DCM phases. The DCM layer was passed through a 0.2 μm pore size syringe filter with a nylon membrane and 2 μL was injected into the GC-FID. The identity of the chromatographic peak (Fig. 2.3C) was confirmed by comparison of the retention time of the sample with that of the standard (Fig. 2.3A). Nicotine extraction and quantification by GC-FID was adapted from Kaldis et al. [2013]. Analysis of nicotine in the biomass extracts followed the methods described in subsection 2.2.4.

![GC-FID chromatogram of (A) nicotine standard, (B)<1 mm tobacco leaf bio-oil of 300-350 °C at 10 °C/min reactor heating rate produced by MFR and (C) <1 mm tobacco leaf extract.](image)

**Fig. 2.3** GC-FID chromatogram of (A) nicotine standard, (B)<1 mm tobacco leaf bio-oil of 300-350 °C at 10 °C/min reactor heating rate produced by MFR and (C) <1 mm tobacco leaf extract.

**2.2.7 Water content (wt%) determination in the bio-oil by Karl Fischer titration**

Water content (wt%) in 0.1 g bio-oil was determined by Karl Fischer titration using a METTLER TOLEDO volumetric Karl Fischer V20 (METTLER TOLEDO, Switzerland) instrument. AQUASTAR® CombiTitrant 5 and AQUASTAR® CombiSolvent Keto were the component reagent and solvent, respectively, both prepared and D (+) glucose (EMD Biosciences, Canada) with known water to check the system to ensure it was operating properly.
2.2.8 Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the differences between three different heating rate of biomass for the bio-oil yield, nicotine concentration and nicotine recovery (PROC GLM, SAS version 9.2, SAS Institute, Cary, NC, USA). Significant differences between three heating rates for the bio-oil yield, nicotine concentration and nicotine recovery were determined by Tukey’s HSD Test (P<0.05). Two-way ANOVA was used to compare the effect of particle size and pyrolysis temperature on bio-oil yield, the nicotine concentration and nicotine recovery. Significant differences between three particle sizes of biomass and pyrolysis temperatures were determined by Tukey’s HSD Test (P<0.05). The standard error (S.E.) is the standard deviation of the sampling distribution of a statistic, most commonly of the mean:

\[ S.E. = \frac{s}{\sqrt{n}} \]

where, \( s \) is the sample standard deviation and \( n \) is the size of the sample.

Because of the small sample sizes used in this study, the confidence intervals are much larger than suggested by the standard error, which is why the results analysis primarily relied on Tukey’s test.

2.3 Results

There was a significant difference between the bio-oil yield (P=0.0008) produced from 15, 10 and 5 °C/min heating rates of <1 mm biomass (Table 2.2). There was no significant difference between the nicotine concentration of the bio-oil (P>0.05) produced from the different heating rates of <1 mm particle size biomass.
Table 2.2 Percent bio-oil yield, percent nicotine concentration in the bio-oil and percent nicotine recovery for <1 mm tobacco leaf biomass pyrolyzed at different heating rates.

<table>
<thead>
<tr>
<th>Heating rate (°C/min)</th>
<th>Percent bio-oil yield</th>
<th>Percent nicotine concentration</th>
<th>Percent nicotine recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>45±0.57(^b)</td>
<td>2.42±0.04(^a)</td>
<td>98.97±0.18(^a)</td>
</tr>
<tr>
<td>10</td>
<td>48±0.70(^a)</td>
<td>2.41±0.04(^a)</td>
<td>104.64±3.11(^a)</td>
</tr>
<tr>
<td>5</td>
<td>39±0.06(^c)</td>
<td>2.14±0.01(^a)</td>
<td>75.12±0.4(^b)</td>
</tr>
</tbody>
</table>

Each value represents the mean ± (S.E.). Means within a column followed by dissimilar lower case letters are significantly different (P<0.05).

Nicotine concentration is defined by the following equation:

Nicotine concentration in bio-oil = (g nicotine in temperature cuts/g bio-oil in temperature cuts) \( (1) \)

There was a significant difference between the heating rates of <1 mm particle size biomass for nicotine recovery (P=0.0025), where both the 10 and 15°C/min heating rates were greater than at 5 °C/min (P<0.05), but no different from each other (P>0.05).

Nicotine recovery is defined by the following equation:

Nicotine recovery compared to conventional solvent extraction method (to be referred to as “nicotine recovery” only from this point on) = (g nicotine in bio-oil produced from 1 g biomass by pyrolysis/g nicotine in 1 g biomass by solvent extraction) \( (2) \)

The 10 °C/min heating rate was chosen for the study of the effect of particle size as it yielded more bio-oil and as high a nicotine recovery as the 15 °C/min heating rate. The percent bio-oil yields (±S.E.) collected for the ambient to 565 °C pyrolysis range were approximately 48 (±0.7), 48 (±1.2) and 49 (±0.8) for the <1, <0.355 and <0.212 mm tobacco leaf biomasses, respectively (Fig. 2.4).

Two-way ANOVA (Analysis Of Variance) was used to compare the effect of particle size and pyrolysis temperature on bio-oil yield. The ANOVA test provides if there is any
significant difference between the bio-oil yields across all temperature cuts for the same or different particle sizes of biomass. It is Two-way ANOVA since there are two factors: factor 1 is different reactor temperature cuts and factor 2 is different particle sizes of biomass.

The results obtained with different reactor temperature ranges and particle sizes are ranked in groups, and statistically tests indicate that there is no statistically significant difference between the members of each group. The groups are identified with letters on the Figure and are ranked alphabetically from high to low (for instance, in Fig. 2.4 a to g). Specific results may belong to more than 1 group. In Fig. 2.4, one can see that all experiments with different particle sizes always belong to the same group. This means that there is no statistically significant impact of the particle size on yield. Fig. 2.4 also shows that there is a statistically significant impact of reactor temperature on bio-oil yield. For example, there is a statistically significant difference between yields from ambient-200 °C and the 200-250 °C ranges. Although there is no statistically significant difference between the 200-250 °C range and the 250-275 °C range, they do not have the same width (200 to 250 °C is 50 °C and 250 to 275 is 25 °C) and there is more oil produced per °C in the 250-275 °C range.

In Fig. 2.4, for the reactor temperature cut of 300-350 °C, the same lower case letter (f) used on the top of the bars indicates that there is no significant difference (P>0.05) between the bio-oil yield of <1, <0.355 and <0.212 mm particle sizes biomasses. Similarly, for the reactor temperature cut of 350-400 °C, the lower case letter d (and sometimes e) on the top of the <1 mm, <0.355 mm and <0.212 mm particle size biomass bars indicates there is no significant difference between the bio-oil yield across the three different particle sizes biomasses. In contrast, there is a significant difference (P<0.05) between the bio-oil yield for the reactor temperature cut of 300-350 and 350-400 °C across the three different particle sizes of biomass since the letter f is present over the 300-350 °C bars but letters d and e are present over the 350-400 °C bars.

The bio-oil yield was significantly higher for the first temperature cut compared to the other cuts, regardless of the biomass particle size (d.f.=26,27; F=362.51; P<0.0001).
However, the particle size of the biomass did not significantly affect the bio-oil yield within each temperature cut (P>0.05).

![Fig. 2.4 Percent bio-oil yield (± S.E.) in batch pyrolysis cuts obtained from tobacco leaf of different particle sizes. Bars with the same lower case letter (a, b, c, d, e, f and g) indicate no significant difference between the bio-oil yield for the biomass particle size across all temperature cuts (PROC GLM; Tukey’s HSD Test, P>0.05).](image)

The percent nicotine concentrations (±S.E.) in the original tobacco biomass were 1.1 (±0.08), 1.21 (±0.06), and 1.28 (±0.04) for <1, <0.355 and <0.212 mm tobacco leaf biomasses, respectively, as determined by solvent extraction.

Nicotine concentration was measured in the bio-oil temperature cuts only between ambient to 400 °C (Fig. 2.5). The nicotine concentration was highest in the 200-250 °C bio-oil temperature cut (d.f.=17,18; F=340.16; P<0.0001) and the smaller particle size of the biomass significantly increased the nicotine concentration compared to the <1 mm size (P<0.05).
Fig. 2.5 Percent nicotine concentration (± S.E.) in batch pyrolysis cuts obtained from tobacco leaf of different particle sizes.

Most of the nicotine was recovered in the first three temperature cuts (Fig. 2.6). The particle size of the biomass had no significant effect for nicotine recovery for the same temperature cut (d.f.=17,18; F=431.05; P>0.05).
Fig. 2.6 Percent nicotine recovery (± S.E.) in batch pyrolysis cuts obtained from tobacco leaf of different particle sizes.

Plotting nicotine concentration vs. recovery demonstrates the decline in nicotine concentration with the accumulative addition of successively lower nicotine-containing temperature cuts (Fig. 2.7). The first and second highest nicotine concentration bio-oils from the <1 mm particle sizes biomass were in the 200-250 and 250-275 °C reactor temperature cuts, respectively (Fig. 2.5). Therefore, the average nicotine concentration in the bio-oil for the combined 200-250 and 250-275 °C cuts is 4.4% when the cumulative nicotine recovery is 74% (Fig. 2.7). The first, second and third highest nicotine concentration bio-oils were in the 200-250, 250-275 and ambient-200 °C reactor temperature cuts, respectively (Fig. 2.5). Therefore, the average nicotine concentration in the bio-oil for the combined 200-250, 250-275 and ambient-200 °C cuts is 3.6% when the cumulative nicotine recovery is 101% (Fig. 2.7). The <0.212 mm particle size biomass produces a higher nicotine concentrated bio-oil than the other two particle sizes when the concentrated temperature cuts are additively accumulated from high to low nicotine. More than 100% of the nicotine in the original tobacco biomass was recovered by MFR, which suggests that more nicotine could be produced through pyrolysis compared to a single solvent extraction technique. Six to seven percent nicotine concentration was
obtained at 200-250 °C for the selected particle size of biomass but the nicotine concentration decreases to below three percent when all the temperature cuts are mixed together.

![Graph showing nicotine concentration vs. percent nicotine recovery for different particle sizes](image)

**Fig. 2.7** Percent nicotine concentration vs. percent nicotine recovery of different particle size tobacco leaf bio-oil when the highest to lowest concentration of nicotine temperature cuts are additively accumulated.

For the remainder of the study the <1 mm biomass particle size was used since no significant difference in bio-oil yield or nicotine content were evident between the different particles sizes (Fig. 2.4 and 2.6). From the above experimental data it is clear that the MFR operating conditions provide higher nicotine recovery. However, as the challenge remains to increase the nicotine concentration in each temperature cut several techniques were considered. Firstly, the water content was calculated for each cut of bio-oil by using the Karl Fischer instrument. It was determined that the nicotine concentration increases (d.f.=11,12; F=291.47; P<0.0001) when the water content in the bio-oil is theoretically deducted (Fig. 2.8). The water-free bio-oil of the second cut produces nicotine concentration more than 3-times compared to the crude bio-oil for the same cut.
Secondly, nicotine concentration was determined after the water and other low boiling temperature compounds were removed by nitrogen drying and heating at 50 °C from each temperature cut. Nicotine concentration of the ambient-200 °C cut was the highest (84%) when the bio-oil was dried at 50 °C compared to any other cut (Fig. 2.9), and when the ambient-200 °C cut was dried by nitrogen the nicotine concentration was also increased (64%), but not as much as by drying at 50 °C. Nicotine was extracted and purified from bio-oil between ambient and 400 °C, but the concentration was the lowest (approximately 1%) at 275-300 °C for both drying methods (d.f.=11,12; F=586.13; P<0.0001).
When water is removed from the bio-oil no difference in the nicotine recovery was determined (d.f.=23,24; F=290.63; P>0.05) since it was calculated theoretically from water-free bio-oil (Fig. 2.10). Nicotine recovery was always reduced for any temperature cut, approximately 84 or 80\%, when the bio-oil is dried by nitrogen gas or 50 °C, respectively.

As was observed in Fig. 2.9, the first temperature cut produces the very high concentration of nicotine (84%) after drying at 50 °C but the nicotine recovery remains low (23%) (Fig. 2.10).
Fig. 2.10 Percent nicotine recovery (± S.E.) for <1 mm particle size tobacco leaf bio-oil in different temperature cuts.

When the temperature cuts are additively accumulated from highest to lowest concentration of nicotine, the concentration of bio-oil dried at 50 °C is still higher than water-free bio-oil (11 vs. 7%) but the nicotine recovery is lower (80 vs. 105%) (Fig. 2.11).
2.4 Discussion

Our findings have demonstrated that pyrolysis by MFR provides a method for biomass conversion to bio-oil and the separation of target chemicals by selective temperature cuts. The use of conventional solvent extraction methods for valuable chemical recovery either from plant biomass or from bio-oil is expensive, time consuming and has detrimental environmental consequences [Hossain et al. 2015]. In addition, pretreatment of the feedstock and the restriction of the solvent type in the extraction make the technique complicated [Zhao et al. 2014]. The MFR process is more cost-effective and will reduce the waste disposal issue associated with solvent extraction.

Isolation of nicotine through boiling of tobacco leaf would be an option compared to the pyrolysis of the biomass. But the additional benefit of pyrolysis of the tobacco leaf is that besides the nicotine produced from the bio-oil, nicotine-free bio-oil and char are also produced have valuable applications. As tobacco residue naturally contains a high level of nicotine, and the boiling point of nicotine is 247 °C, it is most likely that most of the
nicotine identified in the pyrolysis vapors originates from the volatilization of this compound and not from its generation by thermal reaction [Cardoso and Ataíde 2013]. Based on the reactor operating conditions nicotine can be extracted in bio-oil produced up to 400 °C. Above that temperature even lignin, which is one of the principal components of biomass, degrades and produces higher boiling point compounds [Cardoso and Ataíde 2013, Azeez et al. 2011]. Nicotine was chosen for this study for two reasons. Firstly, it is thermostable when the tobacco leaf is pyrolyzed by MFR. Secondly, it has promising medicinal applications [Jin et al. 2013]. For example, Jin et al. [Jin et al. 2013] demonstrated that nicotine-treated dendritic cells have preventive and therapeutic effects on tumor formation. Nicotine provides useful therapeutic treatment for a variety of cognitive impairments including those found in Alzheimer’s disease, schizophrenia and attention deficit hyperactivity disorder [Newhouse et al. 2012, Levin and Rezvani 2000].

The heating rate (5, 10 and 15 °C/min) of the reactor affected the bio-oil yield and nicotine recovery in the produced bio-oil when <1 mm biomass was pyrolyzed. This means that thermochemical reactions are greatly affected by the heating rate of the reactor. Our findings indicate that the particle size of the biomass has no effect on nicotine recovery, at least for the <0.212 and <1 mm range tested. In addition, using <1 mm particle size biomass is more cost effective because it saves time as well as energy compared to grinding for the <0.212 and <0.355 mm particle sizes. Up to 9% more nicotine was recovered by MFR with the <0.212 mm particle size biomass. Therefore, all the nicotine originally in the biomass can be recovered by pyrolysis, but the concentration in the temperature cuts can only be increased by secondary drying with either nitrogen gas or heating to 50 °C. Differences exist between the various cuts due to the amount of water present, and the amount of nicotine originally separated in the particular cut. For example, the 200-250 °C temperature cut has the highest concentration of nicotine (6%) when the nicotine recovery is 45%. In contrast, when the ambient-200 °C temperature cut is dried at 50 °C, the highest concentration of nicotine (84%) is obtained for the nicotine recovery of 23%. To minimize the nicotine loss and to increase the nicotine concentration in the temperature cut there is evidence that a fractional condensation train downstream
of the batch pyrolyzer could be applied to remove the low boiling point compounds including water from the condenser [Gooty et al. 2014]. Some valuable chemicals survive when the biomass is pyrolyzed at a low temperature in the MFR that do not survive high temperature in a conventional fluidized reactor. Hossain et al. [2011] pyrolyzed organosolv lignin with an MFR as well as with a conventional fluidized reactor, finding that lignin monomers, guaiacol and syringol were thermostable at the low MFR temperatures of 250-300 °C but not with fast fluidized reactor temperatures at 450 or 550 °C. We recovered 100% of the nicotine at ambient-275 °C reactor temperature range through the MFR. The pyrolysis at 275 °C of fast fluidized reactor is difficult. The MFR does not, therefore, need to be operated at high temperature to produce nicotine. This is attractive as undesirable by-products of high pyrolysis temperatures in conventional fluidized reactors at high pyrolysis temperatures are PAHs [Hossain et al. 2015], considered an environmental pollutant [Wu et al. 2011].

Differences between the MFR and conventional fluidized reactor include: a) adjustable heating rates that allow the MFR to increase temperatures from ambient to 565 °C, while pyrolysis occurs only at a set temperature for the conventional fluidized reactor; b) MFR is a batch process while conventional fluidized reactor is a continuous process; c) the MFR bio-oil can be fractionated by using different reactor temperature cuts and d) no fluidizing gas (e.g., nitrogen gas) or bed material (e.g., sand) is required in the MFR [Hossain 2011]. Based on these features a higher concentration of target compound can be collected in a particular temperature cut using an MFR which is not possible in the case of conventional fluidized reactor. A conventional fast fluidized reactor achieved its highest yield, 43.4%, of tobacco leaf bio-oil at 500 °C with a vapor residence time of 5 s [Booker et al. 2010], but this was approximately 5% lower than the yield that the MFR produced with the same biomass pyrolyzed from ambient to 500 °C. Unfortunately, it is not possible to compare the bio-oil quality in terms of nicotine concentration produced from fluidized reactor and MFR since the nicotine concentration in the tobacco leaf bio-oil produced by a fast fluidized reactor was not quantified [Booker et al. 2010a]. Based on the protocol [Kaldis et al. 2013] followed in the present study, more nicotine can be isolated from the biomass bio-oil than from the biomass solvent extraction. It is
speculated that multiple, instead of a single, solvent extractions could increase nicotine yield [Jamin et al. 1997] thus increasing nicotine recovery comparable to the amount recovered by MFR. As our objective was to test a single MFR pyrolysis run in place of one single solvent extraction, we believe our results indicate this is an efficient method of nicotine recovery and is more environmentally friendly. The multiple solvent extractions will recover more nicotine but because greater amounts of solvent are required it becomes more expensive and time consuming than pyrolysis.

2.5 Conclusions

This study developed a process to increase nicotine concentration in the tobacco leaf temperature cuts by optimizing the MFR operating conditions. Nicotine was a practical model chemical for bio-oil recovery as it has a relatively high boiling point (247 °C). We were able to demonstrate which bio-oil temperature cuts contained the higher amounts of nicotine and that these cuts can be greatly concentrated by evaporating water and other volatiles. The rest of thesis will focus on improving the MFR condensation train and applying the MFR pyrolysis to recover other valuable chemicals from agricultural and forestry wastes. A further recommendation would be to scale up the batch MFR into a continuous reactor.

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Chapter 3

3 A two-dimensional pyrolysis process to concentrate nicotine during tobacco leaf bio-oil production

Abstract

Many high-value products are currently isolated from plants using complex, expensive and unsafe solvent extraction processes. Pyrolysis is much cheaper, simpler and safer but it is difficult to recover the desired products from these complex liquid mixtures. The objective of this study was to develop a reactor-condenser chain process that would isolate nicotine by the pyrolysis of tobacco leaf. Tobacco (*Nicotiana tabacum*) leaf, <1 mm particle size, was pyrolyzed at 10 °C/min from ambient to 275 °C using a new form of batch pyrolysis technology, a two-dimensional (2-D) batch mechanically fluidized reactor (MFR). The bio-oil produced was separated over six different reactor temperature cuts (ambient-180, 180-210, 210-230, 230-245, 245-260 and 260-275 °C) and collected in either one of two condensers (one hot and one cold). The hot condenser was immersed in a hot oil bath with a temperature set at 180, 190, 200 or 240 °C. The vapors and gases from the hot condenser were passed through a second, cold condenser chilled in an ice bath and the non-condensable gases from the second condenser were then passed through a cotton demister and exhausted. The 2-D MFR operating conditions were optimized by a two-step process: (1) vapors were generated over the whole range of reactor temperatures (ambient-275 °C) and the hot condenser bath temperature was varied to determine which temperature yielded the highest bio-oil and nicotine; and (2) this hot condenser bath temperature was kept constant while the best reactor temperature cut was determined. In step 1, bio-oil collected with a hot condenser bath temperature of 190 °C produced the highest concentrated nicotine (25%) when compared to 180, 200 and 240 °C but there was no significant difference between the nicotine recovery in the hot condenser whether its temperature was 190 or 180 °C (92 and 90% of the nicotine in the original tobacco leaf, respectively). In step 2, when the hot condenser bath temperature was kept at 190
°C, the bio-oil obtained with a reactor temperature between 260 and 275 °C was determined to have the most concentrated nicotine (56%) while the best bio-oil nicotine recovery (28%) was obtained with a reactor temperature between 230 and 245 °C. Overall, the 2-D pyrolysis process allowed for a total nicotine recovery of approximately 90% and a bio-oil nicotine concentration of 20%. These reactor modifications and optimization indicate that the 2-D pyrolysis process is a significant improvement over existing extraction technology and can be used to recover valuable chemicals from biomass.

**Keywords:** Tobacco leaf; Nicotine; Pyrolysis; Mechanically fluidized reactor; Heated condenser.

### 3.1 Introduction

Agricultural residues have been shown to be an important source of bio-fuel [Vamvuka et al. 2014], chemical feedstocks [Yoshida et al. 2005, Iryani et al. 2014, Imran et al. 2014] and a source of many valuable bioactive chemicals [Santana-Méridas et al. 2012]. Fast pyrolysis of tobacco with the Pd/C catalyst produced a valuable alkaloidal product, nicotyrine (Ye et al. 2015). The highest nicotyrine yield was 2.80 wt% at the pyrolysis temperature of 400 °C and catalyst-to-tobacco ratio of 2. The non-catalytic fast pyrolysis of tobacco produced nicotine in the same study. Liu et al. [2013] studied the pyrolysis characteristics and mechanism of tobacco stem. Fast pyrolysis of tobacco stem was carried out at 400, 500, 600, 700 and 800 °C. The main products were confirmed by GC-MS and the chemicals were identified as furfural, guaiacol, phenol and 2-cyclopenten-1-one. Tobacco leaves were pyrolyzed through a fast fluidized bed reactor. The highest bio-oil yield was 43.4% when the pyrolysis temperature was 500 °C and this temperature bio-oil had the highest pesticidal activity [Booker et al. 2010].

An example of a high value crop that generates under-utilized residues is tobacco. Global tobacco leaf production was about 500 million tons in 2006, but over 20% of the leaf resources are discarded as processing waste and pollutes the environment [Wang et al. 2008, Hu et al. 2015]. The discarded tobacco leaves are still economically valuable
because of the bioactive compounds, including nicotine [Wang et al. 2008]. Nicotine and its metabolites [Liu et al. 2015] have useful pharmaceutical properties, for example, nicotine has been found to reduce the symptoms of experimental autoimmune encephalomyelitis [Gao et al. 2015] and Parkinson’s disease [Quik et al. 2008], and improved memory recognition [Froeliger et al. 2009]. Metabolites produced by *Arthrobacter nicotinovorans* degradation of nicotine to 6-Hydroxy-1-nicotine (6HLN) also improved memory formation and decreased oxidative stress in rats, suggesting memory-enhancing and antioxidant effects [Hritcu et al. 2015]. One study with nonsmokers has found that nicotine enhances task performance (Kumari et al. 2003). Although nicotine has negative effect on human health, improved nicotine recovery from tobacco biomass would increase the availability for pharmaceutical applications for particular diseases.

Currently organic solvents are primarily used for the separation of nicotine from tobacco [Hu et al. 2015, Guo et al. 2010]. In most cases methanol is used which is a good solvent for nicotine extraction [Docheva et al. 2014]. But the solvents used are expensive, hazardous and the process is time consuming to purify the target compounds [Hossain et al. 2015]. Moreover, it is critical to select the appropriate solvent to separate the high purity target compound from the plant biomass [Guo et al. 2010]. In addition, the pretreatment of the biomass is an extra step for better separation of the target compounds through solvent extraction [Mäki-Arvela et al. 2014]. Supercritical CO₂ extraction is another technique to recover the valuable chemicals from the plant biomass but still requires the use of co-solvents [De Melo et al. 2016].

A novel approach is to separate nicotine through thermochemical means, such as pyrolysis, in place of chemical solvent extraction. A recent contribution to pyrolysis technology was the development of a mechanically fluidized reactor (MFR) that increased the recovery of nicotine from tobacco leaves (*Nicotiana tabacum*) [chapter 2 (Hossain et al. 2015a)]. A major issue with the process, however, is that the liquid bio-oil produced by this reactor has a nicotine concentration of only 2%, even when the nicotine recovery was better than for solvent extraction. When the bio-oil collected from MFR was dried at 50 °C, the nicotine concentration could be increased to 11%, but the nicotine
recovery decreased (80%) [chapter 2]. It was speculated that some of the nicotine evaporated or degraded when the bio-oil was heated to 50 °C. By removing the water from the bio-oil, the nicotine concentration can be increased. However, the bio-oil at 100 °C or more is highly unstable and rapidly reacts to produce a solid residue [Bridgwater 2003]. One solution found to separate water and other light fractions from the bio-oil was to use a series of high and low temperature condensers following the pyrolysis reactor [Westerhof et al. 2011, Gooty 2012, Pollard et al. 2012]. Gooty [2012] and Gooty et al. [2014] successfully developed a fractional condensation train for the vapors produced from a fluidized bed reactor. In that study, the temperatures of the condensers were optimized to obtain a nearly water-free (less than 1 wt%) bio-oil in the first two condensers and a water-rich bio-oil in the last condenser. This dry bio-oil fraction has a higher heating value (HHV), slightly better than the ethanol fractional condensation, a promising process to produce high quality fuels.

The objectives of this study were: (1) to develop a pyrolysis-condenser process to extract and purify nicotine from tobacco biomass and (2) to optimize the pyrolysis reactor temperature cuts and condenser temperatures to maximize the nicotine recovery.

### 3.2 Materials and methods

#### 3.2.1 Plants

Wild type tobacco plants (*Nicotiana tabacum*) were grown at the Agriculture and Agri-Food Canada (AAFC) research center, Delhi, Ontario, Canada. Tobacco leaves were dried in an oven at 60 °C and were ground with a Thomas Model 4 Wiley Mill® (Thomas Scientific, Swedesboro, NJ, USA) to ≤1 mm particle size. Nicotine concentration in the dried tobacco leaf was 1.1% [chapter 2].

#### 3.2.2 Chemicals

High performance liquid chromatography (HPLC) grade dichloromethane (DCM) (99.7%) was purchased from Caledon Laboratories Ltd., Canada. Reagent grade sodium
hydroxide (NaOH) (97%) and analytical grade (-)-nicotine (98.7%) were purchased from Sigma-Aldrich, Canada.

### 3.2.3 Bio-oil production

The effect of tobacco leaf particle size and reactor heating rate on bio-oil yield and nicotine recovery were previously studied [chapter 2]. The study found that there are no significant differences between <0.212, <0.355 and <1 mm particle size tobacco leaves in terms of nicotine recovery from the biomass with a 10 °C/min reactor heating rate. It was also determined that most of the nicotine was released below 275 °C. Therefore, the present study will focus on the reactor temperature range between ambient and 275 °C, use <1 mm particle size dried tobacco leaf and operate with a 10 °C/min reactor heating rate.

The MFR was located at the Institute for Chemicals and Fuels from Alternative Resources (ICFAR), Western University, London, Ontario, Canada. It is a batch reactor, thus the biomass was placed in the cylindrical reactor chamber before the experiment. The chamber is 15 cm in diameter and 25.4 cm in height, equipped with a stirrer for better heat transfer (Fig. 3.1). An advantage of the MFR is that it does not require nitrogen or other inert gases to fluidize the biomass, therefore avoiding any dilution of the bio-oil vapors and, thus facilitating condensation. The MFR has two band heaters controlled by a Watlow’s EZ-ZONE® PID controller (St. Louis, MO, USA).

A train of two condensers in series was used (Fig. 3.1). The first, hot condenser was immersed in a hot bath containing heat transfer liquid that was maintained at 180, 190, 200 or 240 °C. The gases and vapors exiting from the first condenser entered a second condenser immersed in an ice bath and the non-condensable gases from the second condenser were then passed through a cotton demister and exhausted. Six different condenser trains were used. The bio-oil was collected with a different condenser train for each of six different reactor temperature cuts (ambient-180, 180-210, 210-230, 230-245, 245-260 and 260-275 °C). The reactor temperature was held for 30 min at the end for each temperature cut. The temperature of the hot bath condenser was changed for each MFR pyrolysis run and each run was completed in duplicate.
Definition of 1-D and 2-D MFR pyrolysis process:

When any bio-oil cut is collected only in a cold condenser then it is termed as 1-D MFR pyrolysis process. When a bio-oil cut is collected in a train of hot and cold condensers then it is termed as 2-D MFR pyrolysis process since the bio-oil cut is fractionated and collected at different condenser temperature instead of collecting in a single cold condenser.

![Batch two-dimensional mechanically fluidized reactor](image)

**Fig. 3.1 Batch two-dimensional mechanically fluidized reactor.**

### 3.2.4 Bio-oil sample preparation for gas chromatography-flame ionization detector (GC-FID)

Bio-oil sample preparation and GC-FID method was described in chapter 2. Each temperature cut of 100 µL bio-oil was weighed and was mixed with 4 mL Milli-Q® water (EMD Millipore, Billerica, MA, USA), 2 mL 10% NaOH and 4 mL DCM. The sample was vortexed for 1 min, sonicated for 5 min, shaken for 10 min and was allowed to settle 3 h for separation of the water and DCM phases. The DCM layer was passed through a 0.2 µm pore size syringe filter with a nylon membrane and 2 µL was injected into the
GC-FID (Hewlett Packard 5890 Series II) and compared with a standard curve for nicotine to obtain the nicotine content in the bio-oil. The GC column was a Phenomenex (ZB-5HT, 30 m × 0.25 mm and film thickness 0.25 µm) (Torrance, CA, USA). The injector temperature was 250 °C and inlet pressure was 7 psi. The oven temperature was initially held for 0.5 min at 50 °C, and then increased at 5 °C/min to 125 °C. Next the temperature was increased at 2 °C/min to 155 °C and held for 8 min. At the final stage the temperature was increased at 25 °C/min to 260 °C and held for 8 min. The detector temperature was 275 °C. The nicotine extraction and quantification method was modified from Kaldis et al. [Kaldis et al. 2013], with the exception that GC-FID and an external standard were added as described by Docheva et al. [Docheva et al. 2014].

3.2.5 Biomass extracts sample preparation for GC-FID

Dried tobacco leaf biomass extract sample preparation for GC-FID was described in chapter 2. Dried tobacco leaf, 1 g, was dissolved in 10 mL Milli-Q® water, 5 mL 10% NaOH and 10 mL DCM in an amber-glass vial vortexed for 1 min and sonicated for 10 min. The sample was further shaken for 30 min and was then centrifuged for 15 min at 514 r.c.f. or 1500 r.p.m. It was then allowed to settle for 3 h for separation of water and DCM phases. The DCM phase was passed through a 0.2 µm pore size syringe filter with a nylon membrane and 2 µL was injected into the GC-FID. Nicotine extraction and quantification by GC-FID was adapted from Kaldis et al. [Kaldis et al. 2013].

Nicotine concentration in the experimental data is calculated by the following equation:

\[
\text{Nicotine concentration in bio-oil} = \frac{\text{g nicotine in temperature cut}}{\text{g bio-oil in temperature cut}}
\] (1)

Nicotine recovery in the experimental data is calculated by the following equation:

\[
\text{Nicotine recovery compared to conventional solvent extraction method} = \frac{\text{g nicotine in bio-oil produced from 1 g biomass by pyrolysis}}{\text{g nicotine in 1 g biomass by solvent extraction}}
\] (2)
3.2.6 Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the differences between four different hot bath condenser temperatures for bio-oil yield, nicotine concentration and nicotine recovery (PROC GLM, SAS version 9.2, SAS Institute, Cary, NC, USA). Significant differences between four different hot bath condenser temperatures for bio-oil yield, nicotine concentration and nicotine recovery were determined by Tukey’s HSD comparison of means Test (P<0.05). One-way ANOVA was used to compare between six different reactor temperature cuts for bio-oil yield, nicotine concentration and nicotine recovery. Significant differences between six different reactor temperature cuts for bio-oil yield, nicotine concentration and nicotine recovery were determined by Tukey’s HSD Test (P<0.05).

3.3 Results

As the objective was to increase the nicotine concentration and recovery through separation based on condenser temperature, the nicotine data presented here is only for the bio-oil collected in the hot condenser (Fig. 3.2).

The best 2-D MFR operating conditions for nicotine concentration and recovery were determined in two steps. In step 1, vapors were collected over the whole range of reactor temperatures (ambient-275 °C) while the hot condenser bath temperature was varied between four temperatures. In step 2, the hot condenser bath was held at the best temperature found in step 1, while six reactor temperature ranges were used.

3.3.1 Selection of optimal hot condenser bath temperature

The hot bath condenser temperature of 240 °C had a significantly lower (d.f.=3,4; F=110.67; P=0.0003) bio-oil yield than the other three condenser temperatures, but the 180, 190 and 200 °C condensers were not significantly different from each other in terms of bio-oil yield (P>0.05) (Fig. 3.2).
Fig. 3.2 Percent bio-oil yield (±S.E.) from tobacco leaf collected in four different hot condensers after pyrolysis between ambient-275 °C. Bars with a different lower case letter indicate a significant difference between the bio-oil yield (One Way ANOVA; PROC GLM; Tukey’s HSD Test, P=0.0003).

The nicotine concentration (25%) in the bio-oil collected in a 190 °C condenser was significantly higher compared to the bio-oil collected with other condenser temperatures (d.f.=3,4; F=394.11; P<0.0001) (Fig. 3.3). There was a significant difference in nicotine concentration between the bio-oils collected from all hot condenser temperatures (P<0.05) and the 190 °C hot condenser nicotine concentration was more than 2.3-times greater than in the 240 °C hot condenser.
Fig. 3.3 Percent nicotine concentration (± S.E.) in the tobacco leaf bio-oil collected in hot condensers at different temperatures after pyrolysis between ambient-275 °C.

There was a significant difference in nicotine recovery between the two lower and the two higher hot condenser temperatures (d.f.=3,4; F=98.68; P=0.0003), but no significant difference between the nicotine recovery in the 180 and 190 °C condensers (90 and 92%, respectively) (P>0.05) (Fig. 3.4). Approximately 36 and 62% less nicotine was recovered when the bio-oil was collected in the 200 and 240 °C condensers, respectively, compared to the bio-oil recovery in the 190 °C condenser. Based on these results, the best condenser temperature for high nicotine recovery and high nicotine concentration was determined to be 190 °C.
Fig. 3.4 Percent nicotine recovered (± S.E.) in the tobacco leaf bio-oil collected in four different hot bath condenser temperature after pyrolysis between ambient-275 °C.

3.3.2 Selection of optimal reactor temperature ranges

There was a significantly higher bio-oil yield collected in the 190 °C condenser for the ambient-180 °C range compared to the other reactor temperature ranges (d.f.=5,6; F=98.73; P<0.0001) (Fig. 3.5).
Fig. 3.5 Percent bio-oil yield (± S.E.) from tobacco leaf collected in the 190 °C hot bath condenser at six different reactor temperature cuts.

There was a significantly higher nicotine concentration with increasing reactor temperature cut (d.f.=5,6; f=1130.71; P<0.0001) except between the ambient-180 and 180-210 °C temperature cuts, which produced the lowest concentrated nicotine (Fig. 3.6). The temperature cut at 260-275 °C produced the highest concentration of nicotine.
Fig. 3.6 Percent nicotine concentration (± S.E.) in the tobacco leaf bio-oil collected in 190 °C condenser at six different reactor temperature cuts.

The bio-oil nicotine recovery was highest (28%) at the 230-245 ºC reactor temperature cut compared to all other reactor temperature cuts (d.f.=5,6; F=440.25; P<0.0001) with the exception of the 245-260 ºC cut (Fig. 3.7).
Fig. 3.7 Percent nicotine recovered (± S.E.) in the tobacco leaf bio-oil collected in the 190 °C condenser at six different reactor temperature cuts.

Fig. 3.8 shows how the nicotine concentration varies with the nicotine recovery. It was obtained by adding liquid cuts of progressively lower concentrations. Approximately 75% of the nicotine can be recovered with an average nicotine concentration of 49% (Fig. 3.8). The nicotine concentration for the reactor temperature cut 260-275 °C is 56% when the nicotine recovery is approximately 21%. Overall, the total nicotine recovery is approximately 90% for an average nicotine concentration of 20%.
Fig. 3.8 Percent nicotine concentration vs. percent nicotine recovered from tobacco leaf bio-oil produced by the 2-D MFR with the hot bath condenser set at 190 °C when the highest to lowest nicotine concentration temperature cuts are additively accumulated.

3.4 Discussion

Whatever the technology used, there is a compromise between the purity of the recovered nicotine and its recovery yield. When operating the reactor over its widest range (ambient-400 °C) and condensing all the vapors in an ice-bath condenser, one can recover 100% of the nicotine present in the original tobacco leaf, but the nicotine concentration of the recovered liquid is only 2% [chapter 2]. Crude post-drying of the recovered liquid at 50 °C increases the nicotine concentration to 28% but a significant fraction of the nicotine is lost during drying, reducing the overall recovery to 80% [chapter 2]. Operating the reactor over a wide temperature range (ambient-275 °C) and using a hot condenser to selectively condense a fraction of the hot vapors exiting the reactor, one can obtain a liquid with a nicotine concentration of 20% and a recovery yield of 90%, for the optimum hot condenser bath temperature of 190 °C. The bio-oil yield in the 190 °C hot
condenser for the ambient to 275 °C of reactor temperature range was approximately 5% when the nicotine recovery was 90%.

Using the 2-D approach, the amount of vapors that condense in the hot condenser is a way to separate a few compounds from the total amount exiting the reactor over a narrow range of reactor temperatures. The nicotine concentration can be raised to 49% with a recovery yield of 75% by sending only the vapors exiting the reactor over the temperature cut of 230-275 °C to the hot condenser maintained at 190 °C. As summarized by Fig. 3.8, this range likely represents the economic optimum since the nicotine concentration can be only slightly increased at the expense of a major temperature cut in recovery, while the recovery can only be slightly improved at the expense of a major drop in nicotine concentration.

The results of the 2-D MFR technology for nicotine extraction from tobacco leaf can be compared to two competing processes: (1) the currently commercially dominant solvent-based processes and (2) the more traditional, continuous, high-temperature pyrolysis:

1. The solvent extraction of nicotine from tobacco waste recovered more than 96% [Hu et al. 2015], however experience shows that solvent extraction methods for valuable chemical recovery from plant biomass are expensive, time consuming and are not environmentally friendly [Hossain et al. 2015]. In the present study nicotine recovery and concentration are less than for the solvent extraction [Hu et al. 2015] but by combining the three reactor temperature cuts (230-245, 245-260 and 260-275 °C) the nicotine recovery was 77%. After producing a new batch of concentrated nicotine bio-oil, it is recommended to use a solvent extraction technique to verify the nicotine concentration for the following batches of bio-oil that will be produced. Therefore, the further advantage of the 2-D process is the reduced requirement for organic solvents for the purification of nicotine. In addition, the non-condensable vapors exiting the condensers and bio-oil from other temperature cuts could be used as bio-fuel [Bridgwater 2003] or other valuable applications, for instance, pesticides [Booker et al. 2010]. The bio-char could be used to produce activated carbon [Morali and Şensöz 2015] or fly ash which could be utilized as soil amendment agents [Vamvuka et al. 2014].
2. The traditional solvent extraction method could be avoided if the chemicals are produced through pyrolysis, for instance, pesticidal compounds in bio-oil produced by bubbling bed reactor [Hossain et al. 2015, Booker et al. 2010a, Bedemthu et al. 2011]. Nicotine can be recovered from the bio-oil of tobacco produced at 500 °C (43.4% yield) through bubbling fluidized bed reactor (Booker et al 2010) but the nicotine concentration in the bio-oil was not studied. The disadvantages of using the bubbling fluidized bed reactor for producing bio-oil containing valuable chemicals are: (1) the difficulty in obtaining highly concentrated target chemicals [Hossain et al. 2015, Booker et al. 2010a] and (2) the production of many toxic and carcinogenic polycyclic aromatic hydrocarbons at the higher pyrolysis temperatures [Hossain et al. 2015]. The advantages of using the 2-D MFR process are: (1) the ability to produce bio-oil at different reactor temperature ranges; (2) a highly concentrated target compound can be isolated [Westerhof et al. 2011] and (3) the MFR does not need bed material (e.g., sand), making it easy to recovery pure char by-product, or fluidizing gas (e.g., nitrogen), facilitating vapors condensation [chapter 2, Cascaiosa et al. 2011].

The challenge is to optimize the MFR operating conditions to obtain a high concentration of the target compound in the recovered bio-oil. This study selected nicotine as a test compound for several reasons: (1) nicotine is heat resistant [chapter 2] and (2) has the potential for application as a drug for different diseases. Nicotine has been found to reduce the symptoms of experimental autoimmune encephalomyelitis [Gao et al. 2015]. Several intermediate metabolites of nicotine can be used by the pharmaceutical industry [Liu et al. 2015]. Improved nicotine recovery from tobacco biomass would increase the availability for pharmaceutical application in memory recognition [Froeliger et al. 2009] and Parkinson’s disease [Quik et al. 2008]. Arthrobacter nicotinovorans degrades nicotine to 6-Hydroxy-1-nicotine (6HLN). 6HLN significantly improved memory and decreased oxidative stress, suggesting memory-enhancing and antioxidant effects [Hritcu et al. 2015].

Besides nicotine, there are many other natural products from tobacco and other Solanaceae plants that have potential applications in medicine or as pesticides, and that
would benefit from a more efficient and cost-effective extraction method. Cotinine, the predominant metabolite of nicotine, reduces depressive-like behavior and memory loss in the restrained mice [Grizzell et al. 2014]. Solanesol is available in tobacco waste and can be synthesized into Coenzyme Q10 (CoQ10), a renowned member of the ubiquinone family which is a vitamin-like nutrient and an essential component of the mitochondrial electron transfer chain [Atla et al. 2014]. A related alkaloid, solamargine, from Solanum surattense Burm. F. (Solanaceae) can be used to treat human hepatocellular liver carcinoma (HepG2) and human lung adenocarcinoma (A549) cell lines [Lu et al. 2011]. The glycoalkaloids α-chaconine and α-solanine isolated by aluminum oxide column chromatography from fresh potato Solanum tuberosum tuber were shown to have anticarcinogenic effects on human cancer cell lines, cervical (HeLa), liver (HepG2), lymphoma (U937) and stomach (AGS and KATO III) cancer cells at the concentration range of 0.1-10 μg/mL [Friedman et al. 2005]. Other promising phytochemicals that might be extracted through the 2-D MFR process include allyl isothiocyanate (AIT), derived from the glucosinolate compound, sinigrin, that is found in Brassicaceae crops (mustard seed). AIT-rich mustard seed powder is a highly promising substance for prevention and treatment of bladder cancer [Bhattacharya et al. 2010] and has great antimicrobial activity [Luciano et al. 2009]. Chlorogenic acids, which are natural antioxidants, can be extracted and purified from Mexican green coffee beans Coffea arabica [Suárez-Quiroz et al. 2014].

The 2-D MFR is the first practical technology to concentrate nicotine from tobacco leaves through pyrolysis. The next challenge will be to apply this new technology to the recovery of other valuable, thermally stable chemicals.

3.5 Conclusions

Agricultural and forestry waste contain many valuable chemicals, including phenolics and alkaloids that have potential value as pharmaceuticals and pesticides. Using the new 2-D MFR process, the alkaloid nicotine was extracted from tobacco biomass bio-oil to a concentration of 56% and recovery of approximately 21%. The method of nicotine separation and purification from tobacco leaf through a 2-D MFR pyrolysis process could
be applied to recover the valuable compounds from other plant biomasses. In addition, the environmental issues created by producing synthetic chemicals from petroleum sources and the use of solvents to extract the chemicals from plant sources could be reduced.

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Chapter 4

4 Application of 1-D and 2-D MFR reactor technology for the isolation of insecticidal and anti-microbial properties from pyrolysis bio-oils

Abstract

Valuable chemicals can be separated from agricultural residues by chemical or thermochemical processes. The application of pyrolysis has already been demonstrated as an efficient means to produce a liquid with a high concentration of desired product. The objective of this study was to apply an insect and microorganism bioassay-guided approach to separate and isolate pesticidal compounds from bio-oil produced through biomass pyrolysis. Tobacco leaf (Nicotiana tabacum), tomato plant (Solanum lycopersicum) and spent coffee (Coffea arabica) grounds were pyrolyzed at 10 °C/min from ambient to 565 °C using the mechanically fluidized reactor (MFR). With one-dimensional (1-D) MFR pyrolysis, the composition of the product vapors varied as the reactor temperature was raised allowing for the selection of temperature range that corresponds to vapors with a high concentration of pesticidal properties. Further product separation was performed in a fractional condensation train, or two-dimensional (2-D) MFR pyrolysis, thus allowing for the separation of vapor components according to their condensation temperature. The 300-400 °C tobacco and tomato bio-oil cuts from the 1-D MFR showed the highest insecticidal and antimicrobial activity compared to the other bio-oil cuts. The 300-350 and 350-400 °C bio-oil cuts produced by 2-D MFR had the highest insecticidal activity when the bio-oil was collected from the 210 °C condenser. The tobacco and tomato bio-oil had similar insecticidal activity (LC_{50} of 2.1 and 2.2 mg/mL) when the bio-oil was collected in the 210 °C condenser from the 300-350 °C reactor temperature gases. The 2-D MFR does concentrate the pesticidal products compared to the 1-D MFR and thus can reduce the need for further separation steps such as solvent extraction.
4.1 Introduction

Agricultural residues are a source of renewable energy and valuable chemicals that can be potentially recovered. It has been previously demonstrated that plant biomass can be converted into bio-oil through thermochemical conversion in the absence of air, while at the same time targeted chemicals are separated [chapter 2 (Hossain et al. 2015a)]. Earlier studies determined that bio-oil from pyrolyzed crop residues contain compounds with pesticidal activity [Booker et al. 2010, Bedmutha et al. 2011]. The most toxic biomass component when converted to bio-oil was lignin, in terms of the insecticidal and anti-microbial activity [Hossain et al. 2015]. Tobacco leaves and spent coffee ground bio-oils produced by fast pyrolysis using a bubbling bed reactor exhibited anti-microbial activity that was related to phenols and phenol-derivatives [Booker et al. 2010a, Bedmutha et al. 2011]. The phenolic compounds in bio-oil have antioxidant activity as well [chapter 5] an essential part of the human diet [Balasundram et al. 2006]. Mustard straw bio-oil produced through fast pyrolysis exhibited insecticidal activity that was in-part due to fatty acids that were originally in the biomass [Suqi et al. 2014] and bio-oil from greenhouse tomato plants is a potential source of phenolic compounds with insecticidal activity that are also found in tobacco leaves [Cáceres et al. 2015]. Wood vinegar and other plant extract can be used as biological pesticide or repellent [Fagernäs et al. 2012]. The development of a new pyrolysis technology, the batch mechanically fluidized reactor (MFR), enabled the separation of insecticidal compounds from lignin biomass over several lower temperatures ranges than the single high temperature set on the bubbling bed fast pyrolysis reactor. The MFR temperature range was further optimized to produce a bio-oil cut with the highest insecticidal activity [Hossain et al. 2011]. The advancements made in the previous chapters demonstrate that the 1-D and 2-D MFR could potentially be applied to further concentrate and recover components from the biomass during pyrolysis, regardless of whether the active compounds are previously known.
The tobacco leaves, tomato plant residue and spent coffee ground biomasses were selected for this study in part because they are readily available and because of previous studies that indicated they can be used to produce bio-oil with a range of pesticidal activities. The objective of this study was to isolate bio-oil fractions with a high pesticidal activity without the requirement for further liquid-liquid extraction or chromatographic separation [Hossain et al. 2015].

### 4.2 Materials and methods

#### 4.2.1 Insects, spider mite, bacterial and fungal colonies

The Colorado potato beetle (CPB) (*Leptinotarsa decemlineata* Say) is an insect defoliator of potatoes and it has ability to develop insecticide resistance [Alyokhin et al. 2008]. Cabbage looper (CL) (*Trichoplusia ni* Hübner) is a destructive pest of various crops including *Brassica* crops [Cervantes et al. 2011]. Two-spotted spider mite (TSSM) (*Tetranychus urticae* Koch) is a major pest of many agricultural and ornamental crops across the world [Barati and Hejazi 2015].

Soilborne pathogens of potato include *Rhizoctonia solani* (RS), *Pythium ultimum* (PU) and *Sclerotinia sclerotiorum* (SS) [Larkin and Griffin 2007]. *Clavibacter michiganensis* subsp. *michiganensis* (CM) is one of the most damaging plant pathogens for crops and reduces yields [Kotan et al. 2014]. *Xanthomonas campestris* pv. *vesicatoria* (XC) causes bacterial spot disease on tomato plants and pepper [Bartetzko et al. 2009].

The two insect species (CPB 1st instar larvae and CL 2nd instar larvae), adult TSSM, three fungal species (RS, PU and SS) and two bacterial species (CM and XC) were maintained at the laboratories of Agriculture and Agri-Food Canada (AAFC), London, Ontario, Canada as described in Booker et al. [2010] and Hossain [2011].
4.2.2 Biomass and chemical sources

Tobacco leaves were harvested from AAFC, Delhi, Ontario, Canada and were dried at 60 °C in an oven. The dried biomass was ground to <1 mm particle size and was kept at -20 °C in a freezer until it was pyrolyzed. Tomato plant residues were collected from a greenhouse at Leamington, Ontario, Canada. Coffee (Coffea arabica) grounds (Van Houtte® (Montreal, Quebec, Canada), light roasted, after coffee extraction in a hot water filter was collected from AAFC, London, Ontario, Canada. The pretreatment of tomato plant residues and spent coffee grounds before pyrolysis were the same as tobacco leaves. High performance liquid chromatography (HPLC) grade acetone was obtained from Caledon Laboratories Ltd., Georgetown, Ontario, Canada. Reverse osmosis (RO) water was obtained from AAFC, London, Ontario, Canada.

4.2.3 Bio-oil production

All three biomasses were dried and ground to <1 mm particle size. The ground tobacco leaves, tomato plants and spent coffee were pyrolyzed at 10 °C/min from ambient to 565 °C using the 1-D and 2-D MFR located at the Institute for Chemicals and Fuels from Alternative Resources (ICFAR), Western University, London, Ontario, Canada. As described in chapters 2 and 3 no inert gas or liquid was used to fluidize the biomass during the pyrolysis process in the reactor, rather a stirrer continuously stirred the biomass to improve the heat transfer. The reactor is cylindrical, 15 cm in diameter and 25.4 cm in height. It has two band heaters controlled by a Watlow’s controller (St. Louis, MO, USA). The biomass was placed in the reactor before the experiment as it is a batch process.

For the 1-D MFR experiments, the vapors from the MFR reactor were condensed using stainless steel condensers held in an ice bath (cold temperature condenser) and exiting non-condensable gas was then passed through a cotton demister, to capture any liquid droplets, and exhausted. The condensers for 1-D MFR [Fig. 2.1 in chapter 2] were switched according to the reactor temperature. The bio-oil was collected for four different reactor temperature cuts (ambient-200, 200-300, 300-400 and 400-565 °C). When the
reactor temperature reached the end of a specified temperature interval, i.e. 200, 300, 400 or 565 °C, the reactor temperature was held constant for 30 min. Two batches of each biomass were pyrolyzed to produce the bio-oils.

For the 2-D MFR experiments, the first condenser (hot condenser) of the 2-D MFR setup [Fig. 3.1 in chapter 3] was immersed in a hot bath containing heat transfer liquid that was maintained at the selected temperature (170, 190 or 210 °C in this study). The gases and vapors exiting from the first, hot condenser entered a second condenser (cold condenser) immersed in an ice bath and the non-condensable gases exiting from the second condenser were then passed through a cotton demister and exhausted. The condensers were switched according to the reactor temperature. The bio-oil was collected for two different reactor temperature cuts (300-350 and 350-400 °C). The reactor temperature was held for 30 min at the end of the 400 °C temperature cut. The temperature of the hot bath condenser was changed for each MFR pyrolysis run and each run was completed in duplicate.

4.2.4 Bio-oil sample preparation

Bio-oil collected from the condenser was dried for 3 h under a flow of nitrogen gas at room temperature and was dissolved in 70% acetone (30% of the solvent was water) to prepare solutions at different concentrations. Bio-oil mixtures were prepared by combining all bio-oil cuts from the same biomass proportionally on the basis of their respective yields.

4.2.5 Insect and spider mite bioassays

The CPB 1st instar larvae bioassay was modified from Hossain et al. [2013]. Suppose 3 mg/mL bio-oil solution was used for the insect or TSSM bioassay. It means 3 mg dried bio-oil was mixed with the solvent (70% acetone + 30% water) and the total volume of the solution was 1 mL. The 150 µL or different volume from the solution was taken depending on the method of the assay. Briefly, potato (Solanum tuberosum var. Kennebec) leaf disks were cut to a diameter of 4 cm from fresh potato leaves. The disks were treated with control (70% acetone and 30% water) or dried bio-oil solution (solvent:
70% acetone and 30% water on both sides with a total volume of 150 µL and allowed to dry for 1 h. Leaf disks were placed in 5 cm diameter Gelman Petri dishes on top of Whatman filter paper. Five CPB 1st instar larvae were placed on each treated disk with 3 replicate disks per treatment. The trials were repeated 3-times, and trials to determine the median lethal toxicity (LC$_{50}$) used at least five concentrations of each bio-oil. The mortality of larvae was recorded after 48 h. The CL 2nd instar larval bioassay was modified from Hossain et al. [2015]. The CL leaf disk bioassay is similar to the CPB bioassay with the exception that cabbage (Brassica oleracea var. Golden acre) leaf disks were treated on both sides with a total volume of 100 µL for control or dried bio-oil solution treatments. The procedure for the TSSM leaf disk bioassay is similar to the CPB and CL bioassay with the exception that bean leaf (Phaseolus vulgaris L.) disks were treated on both sides with a total volume of 20 µL for control or dried bio-oil solution treatments and allowed to dry for 20 min [modified from Camara et al. 2015]. Acetone used as a solvent for the bio-oil had no acute toxic effect on the insect or TSSM.

### 4.2.6 Microbial bioassays

The microbial bioassay was adapted from Hossain et al. [2015]. Briefly, a 96-well microtiter plate bioassay was used to test the effect of the bio-oils on the growth of the two bacteria and three fungal species. The 3 mg dried bio-oil/mL solution was tested with bacteria and fungi to observe the microbial inhibition effect. Each well was filled with 100 µL of control or bio-oil solution. Sterile liquid growth medium based on potato dextrose broth (PDB) was used to prepare controls and dilute bio-oils designated as treatments. After inoculation, the 96-well plates of bacteria and fungi were incubated at 21 ºC in a clean culture incubator. The incubation time for bacteria was 3-5 days and for fungi it was 5-7 days. Each control and treatment had three replicates and each experiment was repeated twice. All bio-oil wells were compared to control wells to assess the inhibition levels.
4.2.7 Statistical analysis

Probit analysis (SAS version 9.2, SAS Institute, Cary, NC, USA) was used to determine the LC$_{50}$ values (mg/mL). An LC$_{50}$ is the lethal concentration required to kill 50% of the population. For instance, 150 µL of dried bio-oil solution at 20 mg/mL kill 50% of the total number of insects that have been assayed. Significant differences between LC$_{50}$ values (mg/mL) were agreed on when 95% fiducial limit (F.L.) did not overlap. The F.L. is the boundaries within which a parameter (for instance in this case LC$_{50}$ value) is considered to be located.

Two-way ANOVA was used to compare the effect of different biomasses and pyrolysis temperature on raw/dried bio-oil yield. Significant differences between different biomasses and pyrolysis temperatures were determined by Tukey’s HSD Test (P<0.05). Two-way ANOVA was used to compare the effect of different biomasses and pyrolysis temperature on insect mortality. Significant differences between different biomasses and pyrolysis temperatures were determined by Tukey’s HSD Test (P<0.05).

4.3 Results

The tobacco biomass raw bio-oil yield in the 200-300 °C reactor temperature range was significantly higher at 23% compared to any other cuts or biomass (d.f.=11,12; F=138.99; P<0.0001) (Fig. 4.1). The tomato biomass raw bio-oil yield was highest at 19% in the 200-300 °C reactor temperature range compared to the other cuts (P<0.05). In contrast, the coffee biomass raw bio-oil yield was highest, 18%, in the 300-400 °C reactor temperature range compared to the other cuts (P<0.05) which was 36% of the total bio-oil (Fig. 4.1). The tobacco and tomato biomass dried bio-oil yields were both 8% and were highest in the 400-565 °C reactor temperature range compared to the other dried cuts (d.f.=11,12; F=62.15; P<0.05) and represent approximately 52-53% of the total dried bio-oil (Fig. 4.1). The coffee biomass dried bio-oil yield was 6% also highest in the 400-565 °C reactor temperature range compared to the other cuts of the same biomass (P<0.05) representing 51% of the total bio-oil (Fig. 4.1).
Fig. 4.1 Tobacco, tomato and coffee raw and dried bio-oil yield produced from 1-D MFR. Bars with the same upper case or lower case letter indicate no significant difference between the raw or dried bio-oil yield for the biomass across the temperature cut (Two-way ANOVA, PROC GLM, Tukey’s HSD Test, P>0.05). raw: raw bio-oil; dried: dried bio-oil.

The 30 mg/mL treatment solution made with the 300-400 °C tobacco bio-oil cut produced the highest CPB mortality at 87% (d.f.=11,60; F=21.12.16; P<0.0001) (Fig. 4.2), but there was no significant difference between the mortality of CPB with 300-400 °C tomato bio-oil (73%) cut (P>0.05). The coffee bio-oil cuts produced less than 20% CPB mortality. The CL mortality was no greater than 20% for any tobacco bio-oil cut, and in general the CL was not affected by tomato or coffee bio-oil cut. For this reason, statistical analysis of the CL mortality with all biomass bio-oil cuts was not performed. All tobacco leaf bio-oil cuts produced 100% TSSM mortality at 30 mg/mL (Fig. 4.2). TSSM mortality was 100% for 300-400 and 400-565 °C tomato plant bio-oil cuts, significantly higher than for the other two bio-oil cuts (d.f.=11,60; F=27.19.16; P<0.0001). The 300-400 and 400-565 °C coffee bio-oil cuts were the most active for TSSM mortality at 80%, but not significantly different from the mortality produced by the 200-300 °C bio-oil cut (Fig. 4.2).
Fig. 4.2 CPB, CL and TSSM mortality with 30 mg/mL tobacco leaf, tomato plant residue and spent coffee bio-oil cuts produced from 1-D MFR. Bars with the same upper case or lower case letter indicate no significant difference between the mortality for the same insect across the reactor temperature cuts (Two-way ANOVA, PROC GLM, Tukey’s HSD Test, P>0.05). t: tobacco; tm: tomato; c: coffee.

The 0.3 mg/mL 300-400 °C tobacco leaf bio-oil cut inhibited growth of the bacteria CM and XC (Table 4.1). However, the tobacco bio-oil had no anti-fungal activity against PU, RS and SS. The 0.3 mg/mL 300-400 °C tomato bio-oil inhibited the growth of XC, while PU did not grow in either the 200-300 and 300-400 °C cuts. Coffee bio-oil of 0.3 mg/mL did not inhibit the growth of all the bacteria or fungi species tested.
Table 4.1 The 0.3 mg/mL of tobacco and tomato bio-oil activity against bacteria and fungi.

<table>
<thead>
<tr>
<th>Reactor temperature (°C)</th>
<th>Tobacco bio-oil</th>
<th>Tomato bio-oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CM</td>
<td>XC</td>
</tr>
<tr>
<td>ambient-200</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>200-300</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>300-400</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>400-565</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

-: no inhibition; +: inhibition; CM: Clavibacter michiganensis subsp. michiganensis; XC: Xanthomonas campestris pv. vesicatoria; PU: Pythium ultimum; RS: Rhizoctonia solani; SS: Sclerotinia sclerotiorum

Based on the yield and mortality/growth inhibition results with the 1-D MFR bio-oil produced from the three biomasses, it was determined that the CPB were the most appropriate organism to continue the bioassay guided separation and isolation of the pesticidal activity with the tomato and tobacco bio-oils. Several considerations accounted for this decision, including: 1) it was less time consuming to perform the CPB bioassay; 2) the screening of the pesticidal activity indicated the tobacco and tomato bio-oil had distinct temperature cuts where activity was greater and 3) the CPB mortality was low with the 30 mg/mL coffee bio-oil.

The LC$_{50}$s determined with the CPB bioassay for the different 1-D MFR tobacco bio-oil cuts indicated that the toxicity of the 300-400 °C bio-oil cut was the highest, more than 4.2-times greater than that of the ambient-200 °C cut and 2.7-times greater than the ambient to 565 °C cut (Table 4.2). The LC$_{50}$s for the different 1-D MFR tomato plant bio-oil cuts also indicated that the toxicity of the 300-400 °C bio-oil cut was the highest, 4.2-times greater than that of the ambient-200 °C and 2.7-times greater than the ambient to 565 °C cut (Table 4.2).
Table 4.2 Concentration range for LC$_{50}$ value determination, estimated LC$_{50}$ values (mg/mL) and 95% fiducial limit (F.L.) for different 1-D MFR tobacco leaf and tomato plant bio-oil cuts with CPB.

<table>
<thead>
<tr>
<th>Reactor temperature cuts (°C)</th>
<th>Concentration (mg/mL)</th>
<th>LC$_{50}$ (mg/mL)</th>
<th>95% F.L. (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tobacco</td>
<td>tomato</td>
<td>tobacco</td>
</tr>
<tr>
<td>ambient-565$^a$</td>
<td>35-49</td>
<td>36-50</td>
<td>40</td>
</tr>
<tr>
<td>ambient-200</td>
<td>50-70</td>
<td>63-72</td>
<td>63</td>
</tr>
<tr>
<td>200-300</td>
<td>24-32</td>
<td>35-45</td>
<td>28</td>
</tr>
<tr>
<td>300-400</td>
<td>10-21</td>
<td>14-23</td>
<td>15</td>
</tr>
<tr>
<td>400-565</td>
<td>20-28</td>
<td>22-32</td>
<td>24</td>
</tr>
</tbody>
</table>

$^a$ Bio-oils produced by combining individual bio-oil cuts (ambient-200, 200-300, 300-400 and 400-565 °C) on the basis of their respective yields.

Based on a combination of the dried yield and the LC$_{50}$ value calculated for each tomato and tobacco bio-oil, the 400-565 °C bio-oil cut produced the highest amount of insecticidal compounds compared to the other bio-oil cuts since the bio-oil cut would control the highest number of CPB (Table 4.3).
Table 4.3 The estimated number of CPB controlled by different 1-D MFR tobacco leaf and tomato plant bio-oil cuts were collected in cold condenser and assessed under bioassay conditions.

<table>
<thead>
<tr>
<th>Reactor temperature cuts (°C)</th>
<th>Dried bio-oil (g)</th>
<th>LC&lt;sub&gt;50&lt;/sub&gt; (mg/mL)</th>
<th>Number of CPB killed&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tobacco</td>
<td>tomato</td>
<td>tobacco</td>
</tr>
<tr>
<td>ambient-565&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.62</td>
<td>7.97</td>
<td>40</td>
</tr>
<tr>
<td>ambient-200</td>
<td>0.36</td>
<td>0.26</td>
<td>63</td>
</tr>
<tr>
<td>200-300</td>
<td>2.44</td>
<td>1.96</td>
<td>28</td>
</tr>
<tr>
<td>300-400</td>
<td>0.89</td>
<td>1.52</td>
<td>15</td>
</tr>
<tr>
<td>400-565</td>
<td>3.93</td>
<td>4.23</td>
<td>24</td>
</tr>
</tbody>
</table>

<sup>a</sup> Bio-oils produced by combining individual bio-oil cuts (ambient-200, 200-300, 300-400 and 400-565 °C) on the basis of their respective yields.

<sup>1</sup>Calculation for the data from Table 4.3 (for the number of CPB killed when 200-300 °C 1-D MFR tobacco bio-oil was used): Number of CPB killed = (0.50*5 CPB*1000*2.438 mg)/(150 µL bio-oil solution* LC<sub>50</sub> 28 mg dried bio-oil/1000 µL bio-oil solution) = 1451.

Where, 0.50 = 50% of 5 CPB since 50% CPB killed; 1000*2.438 mg is the dried bio-oil obtained from 100 g biomass; 150 µL bio-oil solution was applied to a potato leaf disk; The LC<sub>50</sub> is 28 mg dried bio-oil/1000 µL solution.

It was determined that both the tobacco and tomato 400-565 °C cuts could be effective at controlling the highest number of CPB based on the LC<sub>50</sub> values and yield even though both the tobacco and tomato 300-400 °C bio-oil cuts were the most toxic to CPB (lower LC<sub>50</sub> values). Since the next step was to further increase the concentration of toxic compounds from the 1-D MFR bio-oil cut, the 300-400 °C tobacco and tomato bio-oil cut was selected for further separation by 2-D pyrolysis into either the hot or cold temperature condensers. The selected hot condenser temperatures were 170, 190 and 210 °C and the reactor temperature range was divided into 300-350 and 350-400 °C.
The yield of 300-350 °C tobacco raw bio-oil collected in the cold condenser was 6 and 7-times higher than that in 190 and 210 °C hot condenser, respectively (d.f.=5,6; F=28.20; P=0.0004) (Fig. 4.3), but there was no significant difference between the cold or hot condenser’s bio-oil yield in the same reactor temperature range (P>0.05). The yield of 350-400 °C tobacco raw bio-oil collected in the cold condenser was 4 to 5-times higher than in the corresponding hot condenser (d.f.=5,6; F=76.16; P<0.0001), but there was no significant difference between the cold or hot condenser’s bio-oil yield when the reactor temperature range was 350-400 °C (P>0.05). The tobacco biomass bio-oil yield was approximately 1% when the 300-350 and 350-400 °C bio-oil cut was dried under nitrogen gas.

Fig. 4.3 Tobacco leaf bio-oil yield in three different hot and cold condensers when the 2-D MFR reactor temperatures are 300-350 and 350-400 °C. Bars with the same upper case or lower case letter indicate no significant difference between the raw or dried bio-oil yield, respectively, within each temperature cut (Two-way ANOVA, PROC GLM, Tukey’s HSD Test, P>0.05). raw = raw bio-oil; dried = dried bio-oil.
The yield of 300-350 °C tomato raw bio-oil collected in the cold condenser was approximately 2-times higher than that in the corresponding hot condenser (d.f.=5,6; F=28.20; P=0.0004) (Fig. 4.4). There was no significant difference among the cold or hot condensers dried bio-oil yield when the reactor temperature range was 300-350 °C (d.f.=5,6; F=101.40; P>0.05). The yield of 350-400 °C tomato raw bio-oil collected in the cold condenser was 3-times higher than that in the corresponding hot condenser (d.f.=5,6; F=125.40; P<0.0001). The tomato biomass bio-oil yield was approximately 2% at reactor temperature range 300 to 400 °C when the bio-oil was dried under nitrogen gas.

![Figure 4.4](image_url)

**Fig. 4.4** Tomato plant bio-oil yield in three different hot and cold condensers when the reactor temperatures are 300-350 and 350-400 °C. Bars with the same upper case or lower case letter indicate no significant difference between the raw or dried bio-oil yield, respectively, within each temperature cut (Two-way ANOVA, PROC GLM, Tukey’s HSD Test, P>0.05). raw = raw bio-oil; dried = dried bio-oil.

The CPB mortality at 3 mg/mL with tobacco and tomato bio-oil collected from the cold condensers was low (<14%) within the reactor temperatures ranges of 300-350 and 350-400 °C. Therefore, only the CPB mortality at 3 mg/mL with tobacco and tomato bio-oil collected from the hot condensers was compared (Fig. 4.5). The CPB mortality with the 3 mg/mL 300-350 °C tobacco leaf bio-oil collected in the 210 °C condenser was the
highest compared to any other hot condenser bio-oil (d.f.=5,30; F=16.64; P<0.0001) (Fig. 4.5). The CPB mortality was the lowest with tobacco 350-400 °C bio-oil collected in the 170 and 190 °C condenser. The CPB mortality with the 3 mg/mL 300-350 °C tomato plant bio-oil collected in 190 and 210 °C condenser was higher than with other reactor bio-oil cut collected in any hot condenser (d.f.=5,30; F=9.97; P<0.0001) (Fig. 4.5). No significant differences in CPB mortality were noted with 300-350 °C tomato bio-oil collected in the 190 and 210 °C condensers (P>0.05).

![CPB mortality with 3 mg/mL tobacco and tomato plant bio-oils from three different hot condensers when the reactor temperatures are 300-350 and 350-400 °C. Bars with the same upper case or lower case letter indicate no significant difference between the mortality for the different tobacco or tomato biomass bio-oils, respectively across the reactor temperature cut (Two-way ANOVA, PROC GLM, Tukey’s HSD Test, P>0.05).](image)

Fig. 4.5 CPB mortality with 3 mg/mL tobacco and tomato plant bio-oils from three different hot condensers when the reactor temperatures are 300-350 and 350-400 °C. Bars with the same upper case or lower case letter indicate no significant difference between the mortality for the different tobacco or tomato biomass bio-oils, respectively across the reactor temperature cut (Two-way ANOVA, PROC GLM, Tukey’s HSD Test, P>0.05).

Fig. 4.5 shows that, of the three hot condenser bio-oils (170, 190 and 210 °C), the 210 °C condenser collected the bio-oil with the highest toxicity from both tobacco and tomato pyrolysis. A comparison of the LC50s for the 210 °C tobacco and tomato condenser bio-oils indicated that the toxicity of the 300-350 °C tobacco bio-oil cut was the highest while the toxicity of the 350-400 °C tomato bio-oil cut was the lowest (Table 4.4).
Table 4.4 The estimated LC<sub>50</sub> values (mg/mL) and 95% fiducial limit (F.L.) for the CPB bioassay with different 2-D MFR tobacco leaf and tomato bio-oil cuts collected in 210 °C hot condenser.

<table>
<thead>
<tr>
<th>Reactor temperature cuts (°C)</th>
<th>Concentration range&lt;sup&gt;1&lt;/sup&gt; (mg/mL)</th>
<th>LC&lt;sub&gt;50&lt;/sub&gt; (mg/mL)</th>
<th>95% F.L. (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300-350</td>
<td>2.1-2.6</td>
<td>1.7-2.6</td>
<td>2.1</td>
</tr>
<tr>
<td>350-400</td>
<td>2.5-3.0</td>
<td>2.0-3.0</td>
<td>2.5</td>
</tr>
</tbody>
</table>

<sup>1</sup>The concentration range (mg/mL) used for determining the LC<sub>50</sub> values.

Based on a similar calculation applied in Table 4.3, the bio-oil cuts from the 300-350 and 350-400 °C collected in the 210 °C condenser together from 2-D MFR killed approximately 5-times more CPB compared to 300-400 °C of 1-D MFR tobacco leaf and tomato plant bio-oil cut (Table 4.5).

Table 4.5 The estimated number of CPB controlled by different 2-D MFR tobacco leaf and tomato plant bio-oil cuts collected in the 210 °C condenser and used under bioassay conditions.

<table>
<thead>
<tr>
<th>Reactor temperature cuts (°C)</th>
<th>Dried bio-oil (g)</th>
<th>LC&lt;sub&gt;50&lt;/sub&gt; (mg/mL)</th>
<th>Number of CPB killed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tobacco</td>
<td>tomato</td>
<td>tobacco</td>
</tr>
<tr>
<td>300-350</td>
<td>0.28</td>
<td>0.74</td>
<td>2.1</td>
</tr>
<tr>
<td>350-400</td>
<td>0.33</td>
<td>0.52</td>
<td>2.5</td>
</tr>
</tbody>
</table>

4.4 Discussion

The MFR bio-oil produced from the three biomass was in order of increasing yield, tomato (43%) < tobacco (48%) < coffee (49%). The bio-oil yields from tomato and coffee produced through the 1-D MFR were higher compared to tomato [Cáceres et al. 2015] and coffee [Bedmutha et al. 2011] bio-oil yields of 38 and 44%, respectively, produced through a traditional fluidized bed reactor. The current study also found there was a higher tobacco leaf bio-oil yield produced through the 1-D MFR compared to the
bio-oil yield (48 vs. 43%) produced through a traditional fluidized bed reactor [Booker et al. 2010]. The advantages of the MFR compared to a fluidized bed reactor [Booker et al. 2010, Cáceres et al. 2015, Bedmutha et al. 2011] are that less reactor temperature is required to obtain bio-oil with pesticidal properties and more highly concentrated pesticidal compounds are produced in the bio-oil. In addition to the pesticide bio-oil fractions produced by the MFR, other temperature cuts and char can be used to produce additional valuable chemicals, for instance, antioxidant and activated carbon [chapters 5 and 6].

Since it is expensive and time consuming to purify the pesticidal compounds through a traditional fluidized bed reactor [Booker et al. 2010, Cáceres et al. 2015, Bedmutha et al. 2011], a solution was developed to purify target compounds from the bio-oil mixture [chapter 3] using a series of high and low temperature condensers following the adjustable temperature pyrolysis reactor [Westerhof et al. 2011, Gooty 2012, Pollard et al. 2012]. Gooty et al. [2014] collected bio-oil in the fractional condensation train for the vapors produced from a fluidized bed reactor at a set reactor temperature. In that study, the temperatures of the condensers were optimized to obtain water-free (less than 1 wt%) bio-oil in the first two condensers and a water-rich bio-oil in the last condenser. However, since bio-oil contains a mixture of compounds, it is difficult to optimize the hot condenser temperature to isolate all the potential target compounds. In addition, many compounds are not heat resistant, therefore making the production of these compounds with a traditional high temperature fluidized bed reactor very challenging. To overcome the complexity of bio-oil purification in order to isolate the target compounds, the batch mechanically fluidized reactor (MFR) was used to produce bio-oil to optimize the temperature cuts [chapter 2] and evaluate the post-pyrolysis process to increase the compound recovery (e.g., drying of bio-oil). The next step was to include a hot and cold temperature condenser, coined a batch “2-D” MFR that demonstrated its effectiveness at concentrating nicotine from tobacco biomass further [chapter 3]. In this study we did not only recover the existing compound in the biomass like nicotine in tobacco leaf (chapters 2 and 3); a wide range of new compounds produced through thermal cracking of the biomass were also collected in the 2-D MFR condensers.
It was observed with both the tobacco and tomato 400-565 °C bio-oil cuts, the highest number of CPB could potentially be controlled. This was based on the LC50 values estimated for the different bio-oil cuts, where the most active cuts were within the temperature range of 300-400 °C for both tobacco and tomato bio-oil. Since the goal was to increase the concentration of toxic compounds from the 1-D MFR bio-oil cut the 300-400 °C tobacco and tomato bio-oil cuts were further separated into either the hot or cold temperature condensers. The findings were that the tobacco bio-oil cut of 300-400 °C exhibited the highest CPB toxicity but the lowest dried bio-oil yield (except the ambient-200 °C bio-oil cut) compared to the other cuts for the same biomass. The bio-oil cuts from the 300-350 and 350-400 °C collected in the 210 °C condenser together from 2-D MFR killed approximately 5-times more CPB compared to 300-400 °C of 1-D MFR tobacco leaf and tomato plant bio-oil cut. The tobacco or tomato bio-oil cuts from the 300-350 and 350-400 °C collected in the 210 °C condenser together from 2-D MFR were more toxic compared to the all 1-D MFR bio-oil cuts ranges from ambient to 565 °C in terms of LC50. That means we were able to produce a high concentrated pesticidal compounds using a 2-D MFR whereas the 1-D MFR produced the low toxic compounds. Some compounds in 1-D MFR bio-oil might have antagonism effect for CPB bioassay [Hossain et al. 2013]. The LC50s of 300-350 °C hot condenser tobacco and tomato bio-oil were 2.1 and 2.2 mg/mL, respectively which contrasted with the LC50s of 5.1 mg/mL for organosolv lignin electrostatic precipitator (ESP) bio-oil produced from bubbling bed reactor [Hossain et al. 2013]. Actually it is difficult to compare the toxicity of different biomass bio-oil produced from different types of reactor. Booker et al. [2010] observed more than 40 and 90% CPB mortality with 10% tobacco aqueous and organic bio-oil fraction in solvent, respectively. Cáceres et al. [2015] studied CPB mortality with tomato biomass bio-oil. The studies reported 64.2% CPB mortality with 30 mg/mL of ESP bio-oil when the ESP bio-oil yield was 6.7% at 500 °C pyrolysis. In our study we measured the toxicity of tobacco and tomato bio-oil in terms of LC50. Actually no comparison can be made between the toxicity of the bio-oil produced from the MFR and the bubbling bed reactor since Cáceres et al. [2015] and Booker et al. [2010] did not study the toxicity in terms of LC50.
4.5 Conclusions

This comparison between tobacco leaf, tomato plant and spent coffee ground biomass bio-oil pesticidal activity determined that tobacco and tomato dried bio-oil collected in a 210 °C hot condenser from a 300-400 °C 2-D MFR reactor produced more than 4-times the insecticidal activity in terms of number of CPB killed compared to the tobacco and tomato dried bio-oil collected from a 300-400 °C 1-D MFR reactor. The process by which the biomass bio-oils are produced through 2-D MFR and separated by condenser temperature shows promise for isolating pesticidal compounds from the bio-oil mixture. Further study for instance liquid chromatography-mass spectrometry (LC-MS) is required to identify which active compounds from the tobacco leaf and tomato plant biomass bio-oil contribute to the pesticidal activity.

Acknowledgments

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Chapter 5

5 Application of 1-D and 2-D MFR reactor technology: Antioxidant activities of tobacco leaf, tomato plant residue and spent coffee ground bio-oils

Abstract

To make biomass conversion to fuels and energy more cost-effective, value-added products, including pharmaceuticals, should be produced from agricultural residues. The objective of this study was to isolate antioxidant compounds from bio-oil obtained with biomass pyrolysis. This chapter uses a mechanically fluidized reactor (MFR) that combines batch pyrolysis with fractional condensation to produce a liquid with a high concentration of desired product. With one-dimensional (1-D) MFR pyrolysis, the composition of the product vapors varies as the reactor temperature is raised, and one can select the reactor temperature range that corresponds to vapors with a high concentration of the pesticidal properties. Further product separation of pyrolysis vapors is performed in a fractional condensation train of two-dimensional (2-D) MFR pyrolysis, which separates vapor components according to their condensation temperature. Tobacco leaf (Nicotiana tabacum), tomato plant (Solanum lycopersicum) and spent coffee (Coffea arabica) grounds (<1 mm particle size) were pyrolyzed with a temperature increasing at a rate of 10 °C/min from ambient to 565 °C. The 400-565 °C reactor temperature cuts of all three biomass bio-oils (tobacco 200-300 and 400-565 °C reactor temperature cuts) from 1-D MFR pyrolysis produced the highest amount of antioxidant compounds compared to the other bio-oil cuts. The 400-565 °C reactor temperature cut produced the highest concentration of antioxidant compounds from 2-D MFR pyrolysis of tomato plant when the bio-oil was collected in a condenser maintained at 120 °C. The MFR can be used to isolate antioxidant compounds from tomato bio-oil within the 400-565 °C reactor temperature range and the 2-D MFR system can further concentrate the antioxidant
compounds by controlling the condenser temperature thus providing a useful tool for processing agricultural residues to value-added compounds.

**Keywords:** Tobacco leaf; Tomato plant residue; Spent coffee grounds; Two-dimensional mechanically fluidized reactor; Antioxidant compounds.

### 5.1 Introduction

The application of biomass conversion technology can solve two challenges created by the disposal of residues from modern agricultural practices, disposal and their environmental impacts. This can be achieved through the production of valuable chemicals from this waste [chapter 2 (Hossain et al. 2015a)]. In many countries, plant waste can be found from field crops, greenhouse crops, and processed commercial food products.

Tobacco leaf production has a global production of 500 million tons, based on the 2006 market, but more than 20% of the leaf resources are discarded as waste leading to negative environmental effects [Wang et al. 2008]. The benefits of extracting bioactive chemicals from tobacco leaves [Wang et al. 2008] and pesticidal compounds from tomato plant residue [Cáceres et al. 2015] have been previously studied, specifically flavonoids [Docheva et al. 2014] and compounds with antioxidant properties through solvent extraction techniques [Ru et al. 2012]. The principal tobacco alkaloid, nicotine, and its metabolites also have useful pharmaceutical properties [Liu et al. 2015] for example the attenuation of experimental autoimmune encephalomyelitis [Gao et al. 2015].

In the case of tomato plant residues, these can be derived from the waste leftover from large greenhouse production in many countries. The valuable compounds that can be extracted from this material include lycopene nanoemulsions which exhibit antioxidant activity [Ha et al. 2015]. Similarly, dried tomatoes [Gümüşay et al. 2015] and waste tomato extracts contain carotenoids which partially contribute to the antioxidant activity [Stajčić et al. 2015]. Even tomatoes grown in soils contaminated with heavy metals have high antioxidant activity [Tommonaro et al. 2015].
Coffee grounds are produced in large quantities globally and an application for this material is an alternative to disposal in landfill. The global coffee production was 143.25 million of 60 kg bags in 2014 [International Coffee Organization 2015] resulting in an equally large amount of waste coffee grounds available for valuable product recovery. For example, thermostable compounds extracted from coffee by using an alkali pretreatment included polysaccharides with antioxidant and antimicrobial properties [Ballesteros et al. 2015]. Green coffees of different geographical origin have noted antioxidant activities [Stelmach et al. 2015], but the highest antioxidant activity was observed with light-roasted coffee beans rather than after further roasting or when the beans were unroasted [Cho et al. 2014]. Ground coffee beans post-beverage preparation may still yield antioxidant activity [Ballesteros et al. 2015].

Biomass conversion through thermochemical means, principally pyrolysis, can yield products with antioxidant properties. Several examples include, pyroligneous acid obtained from *Schisandra chinensis* [Ma et al. 2014], Japanese red pine (*Pinus densiflora* Siebold and Zucc.) [Patra et al. 2015] and a phenolic extract (PE) isolated from *Salix viminalis* pyrolysis bio-oil [Ilnicka et al. 2014]. Of the pyroligneous acids produced from walnut shell at three different temperature ranges (90-150, 151-310 and 311-550 °C), the acid collected from the high temperature range showed the strongest antioxidant activity [Wei et al. 2010].

A new pyrolysis technology, the batch mechanically fluidized reactor (MFR), was first applied to produce bio-oil from lignin which was found to have insecticidal properties [Hossain 2011]. In chapters 2 and 3, modifications to the MFR were used to produce more concentrated nicotine bio-oil from tobacco leaves. The main advantage of the 1-D MFR over the more common fast pyrolysis fluidized bed reactor is that the targeted compounds can be concentrated by only collecting the pyrolytic vapors over a narrow range of reactor temperatures. The advantage of the 2-D MFR over the 1-D MFR is that the targeted compounds can be concentrated further with fractional condensation of the pyrolytic vapors, avoiding or greatly reducing further processing using solvent extraction. Since tobacco leaves, tomato plants and spent coffee grounds are readily available in large quantities as a source of biomass with antioxidant properties, the objective of this
study is to separate and isolate the active compounds through 1-D and 2-D MFR pyrolysis.

5.2 Materials and methods

5.2.1 Biomass and chemical sources

Gallic acid, ammonium molybdate and sodium phosphate were obtained from Sigma Aldrich (Oakville, Ontario, Canada). Sulfuric acid was received from VWR (Mississauga, Ontario, Canada). Methanol was obtained from Caledon Laboratories Ltd., Georgetown, Ontario, Canada. Tobacco leaf, tomato plant and spent coffee ground biomass was collected and pretreated for pyrolysis as described in Chapters 2 and 4. The proximate and ultimate analyses of tobacco leaf, tomato plant and spent coffee grounds are provided in Table 5.1 adapted from Senneca et al. [2007], Yagmur [2012] and Limousy et al. [2015], respectively.

<table>
<thead>
<tr>
<th>Table 5.1 Proximate and ultimate analyses of tobacco, tomato and coffee.</th>
</tr>
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<tbody>
<tr>
<td>Proximate analyses</td>
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<tr>
<td></td>
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<tr>
<td>Ash</td>
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<tr>
<td>Moisture</td>
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<td>Volatiles</td>
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<tr>
<td>Fixed carbon</td>
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<td>Ultimate analyses</td>
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\textsuperscript{a}: dry ash free; \textsuperscript{b} difference
5.2.2 Bio-oil production

Tobacco leaf, tomato plant and spent coffee ground biomass bio-oil was produced through 1-D and 2-D MFR as described in chapters 2 and 4. The 1-D and 2-D MFR were located at the Institute for Chemicals and Fuels from Alternative Resources (ICFAR), Western University, London, Ontario, Canada.

The single condenser for the 1-D MFR [Fig. 2.1 in chapter 2] was switched according to the temperature of the reactor. The bio-oil was collected for four different temperature cuts (ambient-200, 200-300, 300-400 and 400-565 °C). At the end of each cut (i.e., at reactor temperatures of 200, 300, 400 and 565 °C), the temperature was held for 30 min. Two batches of each biomass were pyrolyzed to produce duplicate bio-oil samples for each biomass type. Two condensers in series were used with the 2-D MFR [Fig. 3.1 in chapter 3], the first was immersed in a hot bath and the second in an ice bath. The hot bath contained heat transfer liquid that was maintained at one of the three selected temperatures (120, 160 and 190 °C). The gases and vapors exiting from the hot condenser entered the second condenser immersed in an ice bath and the non-condensable gases from the second condenser were then passed through a cotton demister and exhausted. The condensers were switched between each reactor temperature cut. The hot bath condenser was tested at each of the three hot condenser temperatures and each run was completed in duplicate.

5.2.3 Bio-oil sample preparation for antioxidant activity

The total antioxidant capacity of each bio-oil was measured by the phosphomolybdenum method adapted from Prieto et al. [1999]. Briefly, the raw bio-oil cut of 0.5-20 mg was dissolved in 1 mL methanol. A 0.1 mL aliquot of the bio-oil solution was mixed with 1 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction solutions were incubated for 90 min at 95 °C and transferred to 96 well plates. The absorption was measured at 695 nm using a PowerWave™ XS microplate spectrophotometer (BioTek®, Winooski, VT, USA). The
antioxidant activity was expressed as g gallic acid equivalents per g bio-oil based on a calibration curve range of 0-0.8 mg/mL.

The antioxidant activity for both the 1-D and 2-D MFR was defined as:

1. For the 1-D MFR bio-oil cut: g gallic acid equivalents in a bio-oil cut/g bio-oil in a bio-oil cut
2. For the 2-D MFR hot or cold condenser bio-oil cut: g gallic acid equivalents in a hot or cold condenser bio-oil cut/g bio-oil in a hot or cold condenser bio-oil cut

The antioxidant recovery for both the 1-D and 2-D MFR was defined as:

1. For the 1-D MFR bio-oil cut: g gallic acid equivalents in a bio-oil cut/g gallic acid equivalents in all bio-oil cuts
2. For the 2-D MFR hot or cold condenser bio-oil cut: g gallic acid equivalents in a hot or cold condenser bio-oil cut/g gallic acid equivalents in all bio-oil cuts of 1-D MFR

The antioxidant productivity for both the 1-D and 2-D MFR was defined as:

1. For the 1-D MFR bio-oil cut: g gallic acid equivalents in a bio-oil cut/100 g of pyrolyzed biomass
2. For the 2-D MFR hot or cold condenser bio-oil cut: g gallic acid equivalents in a hot or cold condenser bio-oil cut/100 g of pyrolyzed biomass

5.3 Results

The bio-oil yield of tobacco, tomato and coffee produced through the 1-D MFR was reported in Fig. 4.1, chapter 4. The 200-300 °C reactor temperature range produced the highest amount of tomato and tobacco biomass bio-oil, or 44 and 48% of the total bio-oil, respectively. The 300-400 °C reactor temperature range produced the highest amount of spent coffee ground biomass bio-oil, or 36% of the total bio-oil.

The antioxidant capacity (g gallic acid equivalent per g bio-oil) was the highest with the 400-565 °C temperature range for tomato bio-oil compared to any other cuts regardless of the biomass (d.f.=11,12; F=68.65; P<0.0001) (Fig. 5.1). Only a small amount of
Antioxidant activity was produced from the ambient-200 °C bio-oil cut with the tobacco, tomato and coffee biomass bio-oils.

Fig. 5.1 Gallic acid equivalents ± S.E. determined for tobacco, tomato and coffee bio-oil temperature ranges produced by 1-D MFR pyrolysis. Bars with the same lower case letter indicate no significant difference in the gallic acid equivalent concentration for the reactor temperature cuts produced regardless of the biomass (Two-way ANOVA, PROC GLM, Tukey’s HSD Test, P>0.05).

The antioxidant recovery was highest for the 400-565 °C temperature range for tomato and coffee bio-oil compared to the other cuts for the same biomass (d.f.=11,12; F=325.06; P<0.0001) (Fig. 5.2). More than 82% of the total antioxidant activity was recovered in the 400-565 °C cut for the tomato biomass bio-oil.
Fig. 5.2 Percent gallic acid equivalent recovery ± S.E. determined for the tobacco, tomato and coffee bio-oil temperature ranges produced by 1-D MFR pyrolysis.

Of the total antioxidant activity produced, based on gallic acid equivalents from all the reactor temperature cuts, more than 8% was obtained with tobacco, 8% with tomato and 4% with coffee, using the 1-D MFR process to pyrolyze 100 g of biomass (Fig. 5.3).
Based on the antioxidant activity estimated for the different biomass bio-oils and temperature ranges, the most active cut was the 400-565 °C temperature range for the tomato bio-oil (d.f.=11,12; F=194.78; P<0.0001) (Fig. 5.3). The antioxidant activity for the tobacco bio-oil was dispersed throughout all the cuts except the ambient-200 °C range. The antioxidant activity in the coffee ground biomass was the lowest compared to the other two biomasses. Therefore, from a practical point of view, the 400-565 °C tomato bio-oil cut was selected to further increase the antioxidant concentration using the 2-D MFR with the hot condenser temperatures set at either 120, 160 or 190 °C.

The 2-D MFR tomato bio-oil yield collected in the 120 °C condenser was approximately 2 and 3-times more than in the 160 and 190 °C hot condensers, respectively (d.f.=5,6; F=76.57; P<0.0001) (Fig. 5.4).
Fig. 5.4 Tomato bio-oil yield ± S.E. collected in the hot and cold temperature condensers after pyrolysis by 2-D MFR at the 400-565 °C temperature range.

The antioxidant activity was higher in the 120 and 160 °C condenser bio-oils compared to the 190 °C condenser (d.f.=5.6; F=19.22; P=0.0012) (Fig. 5.5). The cold condenser of the corresponding 120 °C condensers collected the bio-oil with the lowest concentrated antioxidant activity relative to the other cold temperature condensers (<0.05), while the 190 °C condenser did not achieve significant separation, since the oils collected in the hot and cold condensers had about the same activity.
The antioxidant activity recovery was the highest in the 120 °C condenser bio-oil compared to the bio-oil in the other hot/cold condensers (d.f.=5,6; F=145.46; P<0.0001) and the recovery decreased with increasing hot condenser temperature (Fig. 5.6).
Fig. 5.6 Percent gallic acid equivalent recovery ± S.E. in the hot and cold temperature condenser tomato bio-oils after pyrolysis by 2-D MFR at the 400-565 °C temperature range.

Fig. 5.6 shows that nearly 80% of the antioxidant that can be produced from by collecting the vapors from the reactor temperature range of 400-565 °C with a condenser hot temperature of 120 °C. Using an even lower hot condenser temperature would not bring significant additional benefits in recovery or activity, while making process control more challenging. The antioxidant was more concentrated in the 120 °C condenser and the cold condenser for the corresponding 190 °C condenser bio-oil compared to the other condenser bio-oils for each 100 g of tomato biomass pyrolyzed (d.f.=5.6; F=31.54; P=0.0003) (Fig. 5.7), and the antioxidant concentration decreased as the hot condenser temperature was increased.
Fig. 5.7 Gallic acid equivalent ± S.E. in the different hot and cold temperature condenser tomato bio-oil cuts produced from 100 g biomass through 2-D MFR at the 400-565 °C temperature range.

5.4 Discussion

The findings of this research are novel in that it focuses on the production, separation and concentration of compounds associated with antioxidant activity from biomass bio-oil. Since we did not study the antioxidant compounds in biomass before pyrolysis, we do not know whether the antioxidant activity in bio-oil is greater than that of original biomass.

Flavonoids, which have the C₆-C₃-C₆ structure, account for more than half of the over eight thousand different phenolic compounds. The phenolic compound’s antioxidant property depends on the structure, in particular the number and positions of the hydroxyl groups and the nature of substitutions on the aromatic rings [Balasundram et al. 2006]. One study demonstrates that cyclophosphamide (CPA) administration contributes to the induction of oxidative stress in rat brain both in the cerebrum and the cerebellum and that treatment with gallic acid (3, 4, 5-trihydroxybenzoic acid) reversed the oxidative status by increasing the antioxidant defense system [Oyagbemi et al. 2016]. Gallic acid is one of the most important polyphenolic compounds in plants [Eslami et al. 2010]. Gallic acid
and other polyphenolic compounds have been shown to have antioxidant effects [Lu et al. 2006] including nephro and anti-inflammatory effects [Priscilla and Prince 2009]. The pyrolyzed biomass bio-oil contains many phenolic compounds [Bedmutha et al. 2011, Booker et al. 2010] and has measurable antioxidant properties comparable to gallic acid.

The 200-300 °C temperature range produced the highest yield of tobacco and tomato biomass bio-oil. The antioxidant activity of tobacco and tomato was highest, 40 and 82%, respectively, in the 400-565 °C temperature range. In contrast, the highest yield of the spent coffee ground biomass bio-oil was collected in the 300-400 °C temperature range, but the antioxidant recovery was similarly highest in the 400-565 °C temperature range. These results are comparable to those from a study that applied a vertical pyrolytic retort equipped with water cooled condensers to collect walnut shell pyroligneous acids at 90-150, 151-310 and 311-550 °C [Wei et al. 2010]. The acid collected from the highest temperature range had the strongest antioxidant activity and the majority of components in the high temperature range acid were phenols whose concentration was correlated with the antioxidant activity. The biomass bio-oil contains phenolic compounds [Bedmutha et al. 2011, Booker et al. 2010] one example being gallic acid. The antioxidant activity of plant extracts is proportional to the content of phenolic compounds and the gallic acid can be used as a standard to measure the antioxidant activity [Alonso et al. 2016] as the antioxidant activity can be reported in gallic acid equivalents.

In this study less than 4 g of gallic acid equivalent was produced from the 100 g of spent coffee grounds. To our knowledge this is the first study of antioxidant quantity in coffee biomass bio-oil, although others have studied the antioxidant activity through the extraction of polysaccharides from spent coffee grounds by using an alkali pretreatment with sodium hydroxide at 25 °C [Ballesteros et al. 2015]. The total antioxidant activity of polysaccharides extracted from spent coffee grounds was estimated as 0.19 mg/mL in α-tocopherol equivalents per mL of extract. However, as the yield was not provided, no comparison could be made with the antioxidant quantity between the spent coffee ground biomass bio-oil of the present study. Others have suggested that the type of coffee bean, or the roasting of the coffee bean, might affect the antioxidant quantity in biomass bio-oil [Budryn et al. 2015], but this was not examined in the present study.
When the antioxidant properties of flavonoids and polysaccharides from oven-dried tobacco leaves were evaluated in several *in vitro* systems, it was determined that the flavonoids had much better activity than polysaccharides in scavenging free radicals [Ru et al. 2012]. In this case, the scavenging of hydroxyl, superoxide anion, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radicals, and reducing power were compared to ascorbic acid. As this was a different assay of antioxidant properties than that applied in the present study, the amount of antioxidant activity produced by the pyrolysis of tobacco leaf cannot be compared directly to that from tobacco leaf solvent extraction [Ru et al. 2012].

Two important findings in this study with the most promising biomass, tomato plant residue, were: 1) that the 2-D MFR tomato bio-oil yield collected in 120 °C condenser was approximately 2 and 3-times more than the bio-oil yield collected in the 160 and 190 °C condensers, respectively, and 2) the antioxidant activity in the bio-oil of any hot temperature condenser, with the exception of the 190 °C hot condenser, was always higher than that in the corresponding cold condenser bio-oil. However, even though the antioxidant recovery was the highest in the 120 °C condenser, the combined antioxidant recovery in the 120 °C condenser and its corresponding cold condenser was no different that that of either the 160 or 190 °C condensers and their corresponding cold condensers. Therefore, the condensation temperature did not significantly affect the antioxidant properties of the vapor from the reactor, rather the antioxidant compounds only shifted from hot to cold condenser with increasing hot condenser temperature. In comparison, the antioxidant productivity based on gallic acid equivalents produced in the 400-565 °C 1-D MFR cold condenser was 6.6% of the biomass with an antioxidant activity of 82% of gallic acid equivalents, while the antioxidant production at 400-565 °C in the 2-D MFR 120 °C condenser was only slightly lower, at 6.3% of the biomass, with a significantly higher antioxidant activity of 97%. Therefore, the antioxidant activity was concentrated in the bio-oil with the 120 °C condenser by 15% compared to that of 1-D MFR cold condenser alone with only a 0.3% decrease in the antioxidant production. The present study found 8-times more antioxidant activity compared to one that examined the use of different drying process on tomato biomass [Gümüşay et al. 2015]. The antioxidant
activity of tomato fruit dried by sunlight, oven drying, vacuum oven drying and freeze drying determined the antioxidant equivalent was only 1% of the dry biomass. This indicates the advantage of pyrolysis for concentrating compounds with antioxidant activity relative to simply drying the tomato biomass, despite the differences in using fruit vs. plant material and using other protocols for measuring antioxidant activity.

5.5 Conclusions

Antioxidant compounds were concentrated from tobacco leaf, tomato plant residue and spent coffee ground biomass bio-oils by MFR pyrolysis. The highest production of antioxidant was achieved with the tomato plant residue. With the tomato residue, even though there was a trace amount of antioxidants lost (0.3%) when the 2-D MFR was used instead of the 1-D MFR, the antioxidant concentration was increased from 82 to 97% when the 400-565 °C bio-oil was collected in the 120 °C condenser. Further study is required to identify the antioxidant compounds in the biomass bio-oil by using a gas chromatography-mass spectrometry (GC-MS) and other analytical means. A recommendation for future study would be clinical trials of the bio-oil antioxidants for use in livestock feed. These findings show how to efficiently convert agricultural waste to bioactive compounds for possible food and pharmaceutical applications.

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Chapter 6

6 Comparison of activated carbon from biochar produced by pyrolysis of tobacco leaf and tomato plant residue

Abstract

Agricultural crop residue, if not properly managed can contribute to environmental pollution or require associated costs for safe disposal. One solution for this problem is through the recovery of valuable chemicals from this waste material for potential industrial or pharmaceutical applications. In this study tobacco (Nicotiana tabacum) leaf and tomato (Solanum lycopersicum) plant residues were converted to a biochar by pyrolysis from ambient to 565 °C at a heating rate of 10 °C/min using a batch mechanically fluidized reactor (MFR). Activated carbon was produced from the char at 650, 700, 750 and 800 °C through a jiggled bed reactor (JBR). The adsorptive behavior of the tobacco leaf and tomato plant activated carbon obtained from char under optimum preparation conditions were evaluated using methylene blue (MB) as the model adsorbate. The tomato plant activated carbon produced at 750 °C adsorbed over 24 h the highest amount of MB (155 mg MB adsorbed/g activated carbon) compared to the activated carbon produced from other temperatures. In contrast, the tobacco leaf char produced low quality activated carbon. The tobacco leaf activated carbon produced at 800 °C adsorbed the highest amount of MB (52 mg MB adsorbed/g activated carbon) compared to the activated carbon produced from other temperatures after 24 h. The Langmuir and Freundlich isotherms were found to best describe the adsorption characteristics of the tomato plant activated carbon. The tomato plant activated carbon also adsorbed a higher amount of MB compared to commercial coconut shell activated carbon over 48 h (417 vs. 286 g MB/g activated carbon) at equilibrium when the MB initial concentration range was 500-800 mg/L.
In conclusion, it has been demonstrated that tomato and tobacco crop waste can be used to produce activated carbon which in turn could be applied to remove contaminants from municipal and industrial waste water.

**Keywords:** Agricultural residue, Tobacco leaf, Tomato plant, MFR, Char, JBR, Activated carbon.

### 6.1 Introduction

Agricultural crop residues can be used as a low-value energy resource, burned in the field, or simply discarded to landfill, all of which are not considered to be ideal applications or environmentally sustainable. An alternative solution is to apply thermochemical conversion to yield higher grade bio-oils, combustible gases and in the process separate the valuable chemicals from these waste materials. Chapters 2 [Hossain et al. 2015a], 3, 4 and 5 produced bio-oil from the agricultural waste through pyrolysis and have shown it to be a source of pesticides and other valuable chemicals. Another valuable product derived from pyrolysis is the activated carbon that is converted from the bio-char or char.

Activated carbon was derived from NaOH-pretreated rice husks that were pyrolyzed to biochar, tests indicated good adsorption capacity for methylene blue (MB) in aqueous solutions [Lin et al. 2013] and that the rice husk char was effective for cationic malachite green (MG) removal from an aqueous solution [Leng et al. 2015]. Char produced by liquefaction of sewage sludge at 260-380 °C with methanol, ethanol or acetone as the solvent were found to remove both MG and MB from an aqueous solution [Leng et al. 2015a]. Activation with different chemical agents and ratios strongly affects the characteristics of the activated carbons. For example, activating carbon from carbonized tobacco stems combined with K$_2$CO$_3$ activation and microwave radiation affected both MB adsorption and the yield of activated carbon, and under optimum conditions these were 517.5 mg/g and 16.65%, respectively [Li et al. 2008]. When tobacco plant residue, particle size 0.85-0.425 mm, was activated by K$_2$CO$_3$ and KOH, the activated carbon was used to remove phenols from aqueous solutions [Kilic et al. 2011]. Activated carbon
prepared from flame tree *Delonix regia* pods, by first soaking in concentrated H$_2$SO$_4$ at a 1:1 ratio (wt of raw material/volume of acid) for 48 h and then activating at 160 °C for 6 h, produces a useful adsorbent for the removal of MB from aqueous solution [Ho et al. 2009]. The preparation of charred tomato stem activated carbon involved carbonization of the precursor previously impregnated with ortho-phosphoric acid activating agent [Dasgupta et al. 2015]. After carbonization, the carbonaceous material was washed with ammonia solutions, then with distilled water, before drying. The end product had an adsorption capacity of 41.7 mg/g at 308 K and was found effective for the removal of phenol from aqueous solutions. Tomato waste biomass was impregnated with zinc chloride before carbonization, followed by washing with hydrochloric acid solution and hot distilled water and activation after drying at 105 °C [Güzel et al. 2014]. The isotherm analysis indicated that the maximum Orange II (OII) dye monolayer adsorption capacity was 312.5 mg/g under optimum conditions of variables based on the Langmuir model.

Previous studies by our group investigated activated carbon production from olive residue char through a jiggled bed reactor (JBR) [Colomba 2015]. The olive residue was first pyrolyzed at 475 to 550 °C in the JBR and then activated by carbon dioxide at 800 to 900 °C. The adsorption experiments with the olive residue activated carbon fit the Langmuir isotherms and the adsorption capacity was determined to be approximately 50 mg MB/g activated carbon.

Based on the promising conversion of char to activated carbon by the JBR, the objective of this study was to use the same process to produce activated carbon from tobacco leaf and tomato plant residue char produced with the MFR in chapters 4 and 5. These two sources of biomass were chosen in part due to their availability in Canada and the promising results of the previously mentioned chemical activation studies. In the case of tobacco, world production is high, approximately 500 million tons in 2006, and more than 20% of the leaf resources are discarded as waste [Wang et al. 2008, Hu et al. 2015] with the potential that valuable bioactive and pesticidal compounds can still be recovered during pyrolysis [Wang et al. 2008, chapters 3-4]. In the case of tomato, Canada produced 540 ha of greenhouse tomato with a total production was 268,502 tons in 2011 [Statistics Canada 2011]. Much of the tomato plant material becomes a waste biomass
and can also be extracted to recover pesticidal compounds [Cáceres et al. 2015, chapter 4] and antioxidants [chapter 5]. The additional recovery of char for the conversion to activated carbon would provide additional value for both types of biomass.

6.2 Materials and methods

6.2.1 Biomass and chemical sources

MB was purchased from Caledon (Georgetown, Ontario, Canada). Granular activated carbon from coconut shell (GC 12x40 S) was purchased from General Carbon Corporation (Paterson, NJ, USA). Over 90% of the coconut shell particles were within the range of 1.68 mm-0.42 mm. The source of tobacco leaf and tomato plant biomass and pretreatment conditions for pyrolysis were provided in chapters 2 and 4. Tobacco leaves were harvested from AAFC, Delhi, Ontario, Canada. Tomato plant residues were collected from a greenhouse at Leamington, Ontario, Canada. Tobacco leaf and tomato plant residue were dried in an oven at 60 °C and were ground with a Wiley Mill to <1 mm particle size.

6.2.2 Char production

Tobacco leaf and tomato plant residue were pyrolyzed to produce char using a batch mechanically fluidized reactor (MFR). The reactor was described in chapter 2. The reactor temperature was increased at 10 °C/min from ambient to 565 °C with a holding time of 30 min when the reactor temperature reached 200, 300, 400 and 565 °C. We optimized the reactor operating conditions to produce a high yield of valuable chemicals in the bio-oil. The char was obtained as a by-product. The char was collected from the reactor at the end of the pyrolysis and weighed. More than 3 batches of char were collected from tobacco leaf and tomato plant residue.

6.2.3 Activated carbon production through jiggled bed reactor

Char from tobacco leaf and tomato plant residue were activated at the selected temperature using the JBR located at the Institute for Chemicals and Fuels from
Alternative Resources (ICFAR), Western University, London, Ontario, Canada. The JBR (Fig. 6.1) is equipped with an induction heater regulated by a Watlow’s controller (St. Louis, MO, USA). Five min was required for the reactor to reach the selected activation temperature from the ambient temperature. Carbon dioxide at 190 mL/min flow rate was used to activate a 10 g char sample in the reactor for 1 h.

6.2.4 Optimization of the activated temperature to maximize MB adsorption

MB is a typical cationic dye and was selected as the model organic pollutant to study the adsorption characteristics of activated carbon and char. The activated carbon was produced from char at 650, 700, 750 or 800 °C. A 50 mg of activated carbon or char (control) was added to a 50 mL BD FALCON™ polypropylene conical tube (BD Biosciences, Bedford, MA, USA). A 25 mL sample of 500 mg/L MB was added to the sample in the conical tube and mixed using a wrist hand shaker at 23 °C for 24 h (Wrist
Action™ Shaker, Model 75, Burrell Scientific, Pittsburgh, PA, USA) and then centrifuged at 3000 r.p.m. (2056 r.c.f.) (Beckman GP Centrifuge, Beckman Coulter, Fullerton, CA, USA) for 10 min to separate the suspended solids from the liquid sample. A 200 µL aliquot was transferred to a 96-well plate in triplicate. The adsorption was measured at 665 nm using a PowerWave™ XS microplate spectrophotometer (BioTek®, Winooski, VT, USA). The MB adsorption capacity was determined based on the yield of activated carbon.

6.2.5 Contact time study

In order to determine the equilibrium adsorption capacity, the activated carbon sample with the higher yield was selected for exposure to different contact times with 500 mg/L of MB solution. A 50 mg activated carbon sample was added to 25 mL of MB solution and the color absorption reading was taken at 12, 24, 36, 48 and 72 h.

6.2.6 Equilibrium study

The 500, 600, 700 and 800 mg/L MB solutions were tested for adsorption at 24 and 48 h for the tobacco and tomato activated carbon, respectively. A 50 mg activated carbon sample was added to 25 mL of MB solution for each sample. Activated carbon from commercial coconut shell was tested at the same concentration of MB as an industry standard comparison. The particle size of tobacco, tomato and commercial coconut shell activated carbon was below 0.5 mm.

6.2.7 Statistics

Two-way ANOVA was used to compare the effect of biomass and pyrolysis temperature on percent yield of activated carbon, mg methylene blue adsorbed/g activated carbon. Significant differences between two types of biomasses and pyrolysis temperatures were determined by Tukey’s HSD Test (P<0.05).
6.3 Results

As expected, the tomato plant and tobacco leaf activated carbon yield decreased with activation temperature with highest yield of tomato activated carbon produced at 650 °C (d.f.=7,8; F=46.60; P<0.0001) (Fig. 6.2).

![Graph showing yield of tomato and tobacco activated carbon](image)

**Fig. 6.2** Percent yield of tomato plant and tobacco leaf activated carbon ± S.E. at four different activation temperatures. Bars with the same lower case letter indicate no significant difference in the yield of activated carbon for the activation temperature regardless of the biomass (Two-way ANOVA, PROC GLM, Tukey’s HSD Test, P>0.05).

The tomato plant and tobacco leaf activated carbon produced at 750 and 800 °C, respectively, adsorbed the highest amount of MB at 24 h contact time (d.f.=9,10; F=658.00; P<0.0001) (Fig. 6.3). The tomato plant activated carbon produced at 750 °C adsorbed approximately 3-times more MB compared to the tobacco leaf activated carbon produced at 800 °C. The tomato plant char (control) adsorbed 2-times more MB than tobacco leaf char (control). The tomato plant activated carbon produced at 750 °C had more than 5-times the adsorption capacity compared to tomato plant char (control). The
tobacco leaf activated carbon produced at 800 °C had approximately 4-times more adsorption capacity compared to tobacco plant char (control).

**Fig. 6.3** Average methylene blue adsorption (mg methylene blue adsorbed/g char or activated carbon) ± S.E. in a 24 h contact time by tomato plant and tobacco leaf char and activated carbon produced at four different activation temperatures.

Based on the yield of activated carbon, the adsorption capacity of the activated carbon can be expressed per unit mass of original char. The tomato plant and tobacco leaf activated carbon produced at 750 and 800 °C, respectively, both adsorbed the highest amount of 500 mg/L methylene blue solution compared to the other temperature activated carbons and control (char) at 24 h contact time (d.f.=9,10; F=160.78; P<0.0001) (Fig. 6.4).
Fig. 6.4 Average methylene blue adsorption (mg methylene blue adsorbed/g char or activate carbon) ± S.E. based on activated carbon yield at 24 h contact time by char and tomato plant and tobacco leaf activated carbon produced at four activation temperatures.

The equilibrium study with 500 mg/L MB solution at different contact times was studied for tomato plant and tobacco leaf activated carbons. The activation temperature for tomato plant and tobacco leaf activated carbons was 750 and 800 °C, respectively. The equilibrium, $q_e$ (mg/g), was determined at 48 h for tomato plant and 24 h for tobacco leaf activated carbons (Fig. 6.5). The 48 h contact time for MB adsorption by tomato activated carbon was not significantly different from the other contact times (d.f.=9,10; F=348.07; P>0.05) except 12 h. Adsorption quality of MB by tobacco leaf activated carbon was no different at each contact time (P>0.05).
Fig. 6.5 Average methylene blue adsorption (mg methylene blue adsorbed/g activated carbon) ± S.E. by tomato plant (activation temperature 750 °C) and tobacco leaf (activation temperature 800 °C) activated carbon at five contact times.

When the 500, 600, 700 and 800 mg/L MB solutions were tested for adsorption with tobacco, tomato and commercial coconut shell activated carbon at 24, 48 and 48 h, respectively, the results indicate that when equilibrium, $q_e$ (mg/g), was reached, the MB adsorption by tomato leaf and commercial coconut shell activated carbon was correlated with the increase in the MB initial concentration, $C_0$ (mg/mL) (Fig. 6.6). In contrast, when equilibrium was reached by tobacco leaf activated carbon, the MB adsorption was not affected by the MB initial concentration.
Fig. 6.6 Average MB adsorption by coconut shell, tomato plant and tobacco leaf activated carbon when equilibrium, $q_e$ (mg/g), was reached at different MB initial concentration, $C_0$ (mg/mL).

When the 500, 600, 700 and 800 mg/L MB solutions were also tested for equilibrium adsorption with the tobacco, tomato and commercial coconut shell activated carbon at 24, 48 and 48 h, respectively, the concentration of MB in commercial coconut shell and tomato plant activated carbon, $q_e$ (mg/g), was found to increase with the equilibrium concentration in water, $C_e$ (mg/mL), after equilibrium was reached (Fig. 6.7).
Fig. 6.7 Concentration of MB in water, $C_e$ (mg/L), vs. concentration of MB in coconut shell, tomato plant and tobacco leaf activated carbon, $q_e$ (mg/g), after equilibrium was reached.

The equilibrium concentration in water, $C_e$ (mg/mL), had no effect on the concentration of MB in tobacco leaf activated carbon, $q_e$ (mg/g), when equilibrium was reached (Fig. 6.7). Therefore, for this study, two commonly used isotherms i.e., Langmuir [Güzel et al. 2014] and Freundlich [Güzel et al. 2014] were applied in order to determine which one was the best fit with the experimental equilibrium isotherm data for commercial coconut shell and tomato plant activated carbon only.

The Langmuir model assumes a monolayer sorption onto a surface and no interaction between the adsorbed molecules, even on adjacent sites. The linear forms of the Langmuir (Eq. (1)) adsorption isotherm can be expressed as follows:

$$\frac{C_e}{q_e} = \frac{1}{(q_m K_L)} + \frac{C_e}{q_m}$$  \hspace{1cm} (1)

where $C_e$ is the equilibrium concentration (mg/L), $q_e$ is the amount of MB in adsorbent (mg/g) at equilibrium, $q_m$ is $q_e$ for complete monolayer adsorption capacity (mg/g), and $K_L$ is the equilibrium adsorption constant (L/mg). From the slope and intercept of the straight portion of the linear plot obtained by plotting $C_e/q_e$ against $C_e$ (Fig. 6.8), the
values of the Langmuir isotherm parameters were calculated. The Freundlich model was
developed to present multilayer adsorption on a heterogeneous surface. The linear forms
of the Freundlich (Eq. (2)) adsorption isotherm can be expressed as follows:

\[ \ln q_e = \ln K_F + \frac{1}{n} \ln C_e \]  

where \( K_F \) is the Freundlich constant taken as an indicator of adsorption capacity, and \( \frac{1}{n} \) is a measure of the adsorption intensity. From the slope and intercept of straight portion
of the linear plot obtained by plotting \( \ln q_e \) against \( \ln C_e \) (Fig. 6.9), the values of the
Freundlich constants were calculated. The values of isotherm parameters calculated from
both isotherm models are listed in Table 6.1. The mean squared error (MSE) of Langmuir
and Freundlich isotherm for coconut shell activated carbon is 35.2 and 26.6, respectively
whereas for tomato activated carbon it is 18.5 and 5.1, respectively. The Freundlich fits
the data better than the Langmuir isotherm model based on the MSE when plotted \( C_e \) vs.
\( q_e \) for the experimental and predicted value of \( q_e \) for both coconut shell and tomato
activated carbon (Figs. 6.10 and 6.11). The correlation coefficients \( (R^2) \) of coconut shell
and tomato plant activated carbon were 0.99 and 0.99, respectively, for Langmuir
isotherm. The \( R^2 \) of coconut shell and tomato plant activated carbon were 0.95 and 0.99,
respectively, for Freundlich isotherm. The higher \( R^2 \) experimental data were well
described by both Langmuir and Freundlich isotherms.
Fig. 6.8 The Langmuir linear adsorption isotherm of MB onto coconut shell and tomato plant activated carbon at different concentrations.

Fig. 6.9 The Freundlich linear adsorption isotherm of MB onto coconut shell and tomato plant activated carbon at different concentrations.
Fig. 6.10 The experimental and predicted value of MB in coconut shell activated carbon (mg/g) at equilibrium, $q_e$ vs. the equilibrium concentration, $C_e$ (mg/L) for Langmuir and Freundlich models.
Fig. 6.11 The experimental and predicted value of MB in tomato activated carbon (mg/g) at equilibrium, $q_e$ vs. the equilibrium concentration, $C_e$ (mg/L) for Langmuir and Freundlich models.

Table 6.1 Isotherm parameters obtained for adsorption of MB onto coconut shell and tomato plant activated carbon.

<table>
<thead>
<tr>
<th></th>
<th>Coconut shell activated carbon</th>
<th>Tomato plant activated carbon</th>
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</thead>
<tbody>
<tr>
<td><strong>Langmuir</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$q_m$ (mg/g)</td>
<td>286</td>
<td>417</td>
</tr>
<tr>
<td>$K_L$ (L/mg)</td>
<td>0.150</td>
<td>0.004</td>
</tr>
<tr>
<td>MSE</td>
<td>35.2</td>
<td>18.5</td>
</tr>
<tr>
<td><strong>Freundlich</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_F$</td>
<td>200</td>
<td>13</td>
</tr>
<tr>
<td>$1/n$</td>
<td>0.059</td>
<td>0.512</td>
</tr>
<tr>
<td>MSE</td>
<td>26.6</td>
<td>5.1</td>
</tr>
</tbody>
</table>

The $q_m$ value derived from tomato activated carbon was higher than that from coconut activated carbon, indicating that the tomato activated carbon gave a higher potential of
adsorption capacity towards MB. Lower $K_L$ for tomato activated carbon suggests weak sorption bonds between tomato activated carbon and MB compared to coconut activated carbon.

6.4 Discussion

The relationship between adsorbate and adsorbent can be described by equilibrium adsorption isotherm, usually the ratio between the quantity of adsorbate adsorbed and that remaining in the solution at equilibrium at a set temperature. The distribution of MB between the adsorbent and MB solution at equilibrium is important in establishing the capacity of the adsorbent for the MB [Singh and Rawat 1994]. The adsorption capacity of activated carbon for an adsorbate is usually determined by an adsorption isotherm. In order to characterize the adsorption power of adsorbent, the application of an adsorption technique using a basic dye (MB) solution is inexpensive and a common procedure for investigating the effect of the char activation temperature on the removal of MB from aqueous solutions.

Bituminous coal activated carbon produced by steam activation at 1000 °C for 6 h in a rotary kiln followed by washing and sieving to a 0.1-0.8 mm particle size had an adsorption capacity of 253 mg/g at equilibrium with a 600 mg MB/L solution [Qada et al. 2006]. This was greater than the adsorption capacity of tomato plant activated carbon, <0.5 mm particle size, of 198 mg/g at equilibrium with a 600 mg MB/L solution determined in the present study. It has been demonstrated that pre- and post-treatment of the char or activated carbon can increase adsorptive capacity of the final activated carbon. For example, when the tomato stem was impregnated with the requisite amount of orthophosphoric acid as an activating agent, the equilibrium amount adsorbed per g of adsorbent rose from 5.5 to 32.76 mg/g as the initial concentration increased from 30 to 100 mg/L [Dasgupta et al. 2015]. The $q_m$ value of 41.7 mg/g was estimated by applying the linear form of the Langmuir adsorption isotherm model. In our study the value of $q_m$ was 417 mg/g. However, since a phenol solution was used as an adsorbate instead of MB, no direct comparison can be made.
In another study after which the treated chars were heated to different at a rate of 5 °C/min [Lin et al. 2013]. When activated carbon was produced from NaOH-pretreated rice husks activated between 600 and 850 °C, followed by washing to neutrality with 0.1 M sulfuric acid and deionized water, the adsorption equilibrium studies with concentrations of MB between 50 and 500 mg/L calculated a maximum adsorption, \( q_m \), of 396.4 mg/g at 293 K. Usually the pretreatment of char or activated carbon helps improve the adsorption capacity of adsorbate onto activated carbon. In the present study the value of \( q_m \) using the same isotherm model was 417 mg/g for the initial MB solution range of 500-800 mg/L for tomato plant activated carbon without pretreatment. Another study determined the value of the Langmuir parameter, \( q_m \), for MG removal by sewage sludge char was 49.3 mg/g [Leng et al. 2015a]. In this case, the char was produced by liquefaction of sewage sludge at 260-380 °C with methanol, ethanol or acetone as the solvent and dried at 105 °C for 24 h and ground to less than 75 µm before batch sorption experiment. The initial adsorbate concentration range was 20-120 mg/L. The MG adsorption equilibrium data had an excellent fit to the linear form of Langmuir model.

We did not study the effect of particle size of adsorbate and pH of MB solutions for adsorption of MB onto activated carbon. The particle size of adsorbate and pH plays a very important role for MB adsorption onto activated carbon [Qada et al. 2006]. This study did not use any chemicals for the pre-treatment or post-treatment to improve the adsorption capacity of the tomato plant activated carbon. Adding chemicals to improve the adsorption capacity of the activated carbon is expensive and is not environmentally friendly.

6.5 Conclusions

Tomato plant and tobacco leaf char was produced as the by-product of pyrolysis from the studies described in chapters 4 and 5. When the char was converted to activated carbon it was compared as a function of contact time in order to determine the necessary adsorption equilibrium time with MB, the model organic pollutant. It was found that the tomato plant activated carbon had an adsorption capacity higher than that of commercial coconut shell activated carbon (417 vs. 286 mg/g). With tobacco leaf, on the other hand,
there was no increment of MB to tobacco leaf activated carbon for the MB initial concentration range, the data was not fit to the adsorption isotherm model, and this indicated that tobacco leaf char did not produce a good quality activated carbon. Langmuir and Freundlich isotherms were found to fit the experimental data well for commercial coconut shell and tomato plant activated carbon over the concentration range as indicated by the high correlation coefficients ($R^2$). Of the two isotherm models applied, Freundlich isotherm was found to better fit the experimental data by the low mean squared error (MSE).

One potential application for the tomato activated carbon would be the removal of contaminants from municipal and industrial waste water, normally an expensive process. Since agricultural waste, such as tomato plant residues, is a low cost material, value-added products, including activated carbon for capturing organic contaminants from water, could be obtained in a cost-effective manner using this process.

Acknowledgments

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Chapter 7

7 Application of 1-D and 2-D MFR reactor technology:
Hydrolysis and organosolv lignins

Abstract

Many valuable chemicals can be recovered during thermochemical conversion for fuel production from biomass residues, including those produced from agriculture and forestry wastes. The benefits of the cost recovery include reducing environmental pollution and the dependency on fossil fuel. The objective of this study was to produce antioxidants, pesticides and char from hydrolysis and organosolv lignin through a pyrolysis process. A mechanically fluidized reactor (MFR) was used to produce bio-oil within different reactor temperature ranges so that the antioxidant and pesticide activity could be screened. It was determined that 95 to 99% of the total antioxidant recovery came from bio-oil produced in the 300-400 °C reactor temperature range of the one dimensional (1-D) MFR while the 400-565 °C reactor temperature range yielded the hydrolysis and organosolv lignin bio-oil cut with the highest insecticidal toxicity (LC$_{50}$ = 9.9 mg/mL). When the two-dimensional (2-D) MFR pyrolysis process was used, the antioxidant activity recovery and concentration in hydrolysis lignin bio-oil decreased but the insecticide toxicity increased (LC$_{50}$ = 4.7 mg/mL) compared to 1-D MFR. In contrast, the 2-D MFR pyrolysis process decreased the antioxidant activity recovery whereas increased the antioxidant concentration in the organosolv lignin bio-oil compared to 1-D MFR and the insecticide toxicity increased (LC$_{50}$ = 4.7 mg/mL). The char from hydrolysis and organosolv lignin MFR pyrolysis was activated with carbon dioxide at four different activation temperatures (650, 700, 750 and 800 °C) through a jiggled bed reactor (JBR) to produce the activated carbon. However, no significant adsorption capacity was observed from the hydrolysis and organosolv lignin biomass carbonaceous materials.
The findings indicate that the 2-D MFR process is most promising if used to increase the pesticidal toxicity of lignin wastes, and the concentration of antioxidant compounds from organosolv lignin biomass bio-oil.

**Keywords:** Hydrolysis lignin, Organosolv lignin, MFR, Insecticidal toxicity, Antioxidant activity, Char, JBR, Activated carbon.

### 7.1 Introduction

Lignin is the second most abundant organic natural compound in the world after cellulose. Approximately 50 million tons of lignin are produced yearly worldwide [The International Lignin Institute 2015]. There are two principal categories of lignin: those which are sulfur bearing and those which are sulfur-free. The sulfur containing lignins are produced from kraft and sulphite pulping processes. This first category comprises almost the whole market of commercially available lignins [Silva et al. 2009]. The organosolv and hydrolysis lignin fall into the second category. Due to the lack of suitable industrial processes, the sulfur-free lignins are as yet non-commercialized [The International Lignin Institute 2015].

Lignin is a phenolic high molecular wt biopolymer (600-15000 kDa), composed of a highly branched phenylpropanoid framework. Its three monomers, p-coumaryl-, coniferyl- and sinapyl alcohol vary in abundance depending on the plant species. Lignin accounts for 20% of the total weight of the planet’s biosphere and crude lignin is obtained in large quantities in the pulp and paper industry [Kleinert and Barth 2008]. Currently lignin is used in low value-added applications and for energy production [Santos et al. 2014] but also represents a potential source of valuable chemicals.

Organosolv lignin is produced from the cellulosic ethanol industry, and because of the increase in ethanol as a fuel source, more organosolv lignin is available [Kim et al. 2015]. A sulfuric acid-free ethanol cooking treatment was also developed to achieve complete saccharification of the cellulosic component of eucalyptus and bagasse flour, thereby avoiding the problems associated with the use of strong acid catalysts [Teramoto et al. 2008]. The lignin resulting from organosolv pretreatment of sugarcane bagasse is of high-
quality, primarily unaltered when compared to kraft lignin, and provides enough quality to be recycled and converted into valuable products [Mesa et al. 2016]. Hydrolysis lignin is obtained as a by-product of the cellulosic sugar-based chemical or ethanol industry [Mahmood et al. 2015], but the by-product lignin produced through the enzymatic hydrolysis process is not pure [Mesa et al. 2016]. Since ethanol production has increased in recent years there is a large amount of both organosolv and hydrolysis lignin available as the by-product in bioethanol industries. The kraft process (also sulfate process) is a process for conversion of wood into wood pulp consisting of almost pure cellulose fibers by adding a mixture of sodium hydroxide and sodium sulfide with wood chips. In the kraft pulping process, the lignin-containing by-product is kraft black liquor, and the dry content of weak black liquor is approximately 15%. Lignin, which constitutes approximately 30-45% of the dry material, is the main wood component extracted into the liquor [Helander et al. 2016]. Today, kraft lignin is mainly used as a fuel.

The major pyrolysis reactions of lignin occur between 200 and 600 °C and volatile compounds are produced as lignin undergoes thermal degradation over a wide range of temperature [Wang et al. 2009]. Therefore, it would be efficient to collect the volatiles released during lignin pyrolysis in the fractional condensation train downstream of the reactor. The potential applications for lignin compounds are many; they include pharmaceutical or food preservation (antioxidants) and pest control (insecticides), and separation by the reactor-condenser process could provide a cost-effective lignin biorefinery.

Other processes to separate lignins include the use of acid precipitation of black liquor from kraft pulp using different pH [Santos et al. 2014]. Samples were referenced as follows: lignin precipitated with sulfuric acid at pH 2 LKS 2, at pH 4 LKS 4 and at pH 6 LKS 6. In this case, lignin was precipitated with sulfuric acid (98% w/w) at pH 2, 4 and 6 in combination with LKS 2, 4 and 6. The antioxidant potential determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was the highest for the low pH, LKS 2 treatment. The lignin extracts of the herbaceous species, Moso (Phyllostachys edulis), had DPPH radical-scavenging activity (IC$_{50}$ = 20.5 ± 2.1 mg/mL) that was correlated with the total phenolic content [Kurakake et al. 2015]. In the
preparation of the degraded lignin from Moso, 10 mL distilled water was added and mixed after the treatment with ammonia water. The supernatant containing the degraded lignin was isolated by centrifugation. The lignin solution was neutralized with sulfuric acid at a ratio 3:2 (lignin extract:sulfuric acid), and diluted by Tri-HCl buffer. The pH was adjusted to 7.2-7.5 and the solution tested for its antioxidant activity.

Previous research from our group determined that activated carbon could be produced from olive plant residue and kraft lignin using a jiggled bed reactor (JBR) [Colomba 2015]. The olive residue and kraft lignin were individually heated to the pyrolysis temperature of 475 to 550 °C in the JBR, and the resulting char was activated in the optimal temperature range between 800 to 900 °C. The olive residue activated carbon fit the Langmuir isotherms and the adsorption capacity was determined to be approximately 50 mg MB/g activated carbon, whereas the adsorption capacity for the kraft lignin was 100 mg MB/g activated carbon depending on the mesopore volume of activated carbon (cm³/g). The tomato plant residue char from 1-D MFR pyrolysis was produced at ambient to 565 °C and was carbonized by JBR also produced activated carbon [chapter 6]. The adsorption capacity of tomato activated carbon was determined to be approximately 417 mg MB/g activated carbon. The difference between our group’s studies and those of others is that we did not pre-treat the lignin before pyrolysis. For example, sodium hydroxide-pretreated rice husks when activated had excellent uptake capability towards MB [Lin et al. 2013], and rice husk char was effective on cationic malachite green (MG) removal from aqueous solution [Leng et al. 2015].

When a fast pyrolysis fluidized bed reactor was used to produce the bio-oil from cellulose, hemicellulose and organosolv lignin, the lignin biomass bio-oil produced the highest insecticidal activity [Hossain et al. 2013]. The 48 h LC₅₀ value determined for lignin bio-oil with the larval Colorado potato beetle (CPB) (Leptinotarsa decemlineata Say) bioassay was 7.6 mg/mL, whereas it was 96.9 and 21.7 mg/mL for the cellulose and hemicellulose bio-oils, respectively. It was determined that the organosolv lignin bio-oil produced at 550 °C by a fast pyrolysis reactor also contains polycyclic aromatic hydrocarbons (PAH) which are considered as environmental contaminants and not desirable in the bio-oil production [Hossain et al. 2015]. For this reason the mechanically
fluidized reactor (MFR) was developed so that bio-oil can be produced over a lower range of temperature cuts to avoid PAH production. Instead, the organosolv lignin bio-oil cut from the MFR with the highest insecticidal toxicity contained mainly phenolics, such as guaiacol, catechol and syringol [Hossain 2011].

The advantage of the MFR over fast pyrolysis is that it does not require bed material, except the biomass, or inert gas for the fluidization of the bed. It can also be used to slowly increase the reactor temperature, and bio-oil produced can be separated in a chain of condensers. The MFR temperature cuts were used to improve the separation chemicals and was determined to be less expensive compared to the two-step process for the separation of bubbling bed reactor bio-oil with solvents. The MFR was used to extract nicotine from tobacco leaf biomass [chapter 2 (Hossain et al. 2015a)] but the concentration in the bio-oil was very low due to water and other low boiling point compounds. The modification of the MFR by the addition of temperature-controlled condenser systems allowed for the bio-oil to be collected at specific temperature ranges and increased the concentration of the nicotine [chapter 3]. When the two-dimensional (2-D) MFR was applied to tobacco leaf and tomato plant biomass, pesticide and antioxidant components were separated from the bio-oil [chapters 4-5]. In all cases the higher temperature condenser of the 2-D MFR was observed to remove water and other non-target compounds from the fraction containing the pesticide or antioxidant activity.

As a further proof of concept for the 2-D MFR process, the objective of this study was to optimize the reactor temperature cuts and condenser heating-cooling temperatures to increase the concentration and recovery of fractions containing the highest pesticide and antioxidant activity from hydrolysis and organosolv lignin pyrolysis bio-oil. Another objective of this study was to produce and compare the activated carbon from hydrolysis and organosolv lignin char by-product.
7.2 Materials and methods

7.2.1 Biomass and chemical sources

Hydrolysis lignin (purity 55-57%) with a particle size less than 355 µm was provided by FPInnovations (Pointe-Claire, QC, Canada). Organosolv lignin (purity > 95%) with a particle size less than 200 µm was provided by the Institute for Chemicals and Fuels from Alternative Resources (ICFAR), Western University, London, Ontario, Canada. Gallic acid, ammonium molybdate and sodium phosphate were obtained from Sigma Aldrich (Oakville, Ontario, Canada). Sulfuric acid was received from VWR (Mississauga, Ontario, Canada). High performance liquid chromatography (HPLC) grade acetone and methanol were obtained from Caledon Laboratories Ltd., Georgetown, Ontario, Canada. Methylene blue was purchased from Caledon (Georgetown, Ontario, Canada). Granular activated carbon from coconut shell (GC 12x40 S) was purchased from General Carbon Corporation (Paterson, NJ, USA). Over 90% of the coconut shell particles were within the range of 1.68 mm-0.42 mm. MB was purchased from Caledon (Georgetown, Ontario, Canada). Granular activated carbon from coconut shell (GC 12x40 S) was purchased from Paterson, NJ, USA. A 90% particle of activated carbon from coconut shell was within the range of 1.68 mm-0.42 mm.

7.2.2 Insects, bacterial and fungal colonies

The Colorado potato beetle (CPB) (*Leptinotarsa decemlineata* (Say)) is the most important insect defoliator of potatoes and it develops insecticide resistance quickly [Alyokhin et al. 2008]. Brassica crops have been associated with reductions in soilborne pathogens of potato, including *Rhizoctonia solani* (RS), *Pythium ultimum* (PU) and *Sclerotinia sclerotiorum* (SS) [Larkin and Griffin 2007]. *Clavibacter michiganensis* subsp. *michiganensis* (CM) causes Bacterial canker of tomato (*Lycopersicum esculentum* Mill.). Bacterial canker of tomato is an important disease that damages the leaves and reduces yields [Chang et al. 1992], while *Xanthomonas campestris* pv. *vesicatoria* (XC) causes bacterial spot disease on tomato plants and pepper [Bartetzko et al. 2009].
One insect species (CPB 1st instar larvae), two bacterial species (CM and XC) and three fungal species (RS, PU and SS) were maintained at Agriculture and Agri-Food Canada (AAFC), London, Ontario, Canada as described in Booker et al. [2010] and Hossain [2011].

The CPB were reared on potato plants (*Solanum tuberosum* var. Kennebec) at 25 °C, 50% relative humidity (RH) and 16:8 h light:dark photoperiod. CPB were reared for over 130 generations without exposure to insecticides. Potato plants for the insect rearing and for the insect bioassay were grown in a climate controlled greenhouse. Bacteria and fungi were grown on tryptic soy agar (TSA) medium and potato dextrose agar plates, respectively.

### 7.2.3 Bio-oil production

Hydrolysis and organosolv lignin biomass bio-oil was produced through pyrolysis with the 1-D and 2-D MFR at the Institute for Chemicals and Fuels from Alternative Resources (ICFAR), Western University, London, Ontario, Canada. The 1-D and 2-D MFR operating conditions were described in chapters 2 and 3. Briefly, the single condensers for the 1-D MFR [Fig. 2.1 in chapter 2] were switched according to the reactor temperature. The bio-oil was collected in four different temperature cuts (ambient-200, 200-300, 300-400 and 400-565 °C). At the reactor temperatures of 200, 300, 400 and 565 °C, the temperature was held for 30 min. Two batches of each biomass were pyrolyzed to produce the duplicate bio-oil samples for each biomass type.

The double condensers of 2-D MFR [Fig. 3.1 in chapter 3] were immersed in either a hot or cold bath. Gases exiting the reactor entered the hot condenser partially submerged in a hot bath containing heat transfer liquid that was maintained at one of the two selected temperatures (120 and 150 °C). The gases and vapors exiting from the hot condenser entered the second condenser immersed in an ice bath and the non-condensable gases from the second condenser were then passed through a cotton demister and exhausted. The condensers were changed according to the temperature of the reactor bed. The bio-oil was collected from the hot and cold condensers for the reactor temperature range of 300-400 and 400-565 °C and the reactor temperature was held for 30 min at the end of each
temperature range (i.e., 400 and 565 °C). The temperature of the hot bath condenser was tested at each of the two temperatures and each run was completed in duplicate.

7.2.4 Bio-oil sample preparation for insect bioassays

The insect bioassay was adapted from Hossain et al. [2013]. Briefly, bio-oils were prepared by pyrolysis of hydrolysis or organosolv lignin. Bio-oil collected from the condenser was dried under a flow of nitrogen gas at room temperature and was dissolved in 70% acetone to prepare solutions at different concentrations. Bio-oil mixtures were prepared by combining all bio-oil cuts from the same biomass proportionally on the basis of their respective yields.

7.2.5 Insect bioassays

The CPB 1st instar larvae bioassay was described in Hossain et al. [2013]. Briefly, potato (*Solanum tuberosum* var. Kennebec) leaf disks were cut to a diameter of 4 cm from fresh potato leaves. The disks were treated with 70% acetone (control) or bio-oil on both sides with a total volume of 150 µL and allowed to dry for 1 h. Leaf disks were placed in 5 cm diameter Gelman Petri dishes on top of Whatman filter paper. Five CPB 1st instar larvae were placed on each treated disk with 3 replicate disks per treatment. The trials were repeated 2-times, and trials to determine the median lethal toxicity (LC$_{50}$) used at least five concentrations of each bio-oil. The mortality of larvae was recorded after 48 h.

7.2.6 Microbial bioassays

The microbial bioassay was adapted from Hossain et al. [2015] and described in chapter 4. Briefly, a 96-well microtiter plate bioassay was used to test the effect of the bio-oils on the growth of the two bacteria and three fungal species. Sterile liquid growth medium based on potato dextrose broth (PDB) was used to prepare controls and dilute bio-oils designated as treatments. After inoculation, the 96-well plates of bacteria and fungi were incubated at 21 °C in a clean culture incubator. The incubation time for bacteria was 3-5 days and for fungi it was 5-7 days. Each control and treatment had three replicates and
each experiment was repeated twice. All bio-oil wells were compared to control wells on each plate to assess the inhibition levels.

7.2.7 Bio-oil sample preparation for antioxidant activity

The total antioxidant capacity of each raw bio-oil was measured by the phosphomolybdenum method adapted from Prieto et al. [1999]. Briefly, the raw bio-oil cut of 0.5-20 mg was dissolved in 1 mL methanol. A 0.1 mL aliquot of the bio-oil solution was mixed with 1 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction solutions were incubated for 90 min at 95 °C and transferred to 96 well plates. The absorption was measured at 695 nm using a PowerWave™ XS microplate spectrophotometer (BioTek®, Winooski, VT, USA). The antioxidant capacity was expressed as g gallic acid equivalents per g raw bio-oil based on a calibration curve range of 0.05-0.8 mg/mL.

The antioxidant activity for both the 1-D and 2-D MFR was defined as:

1. For the 1-D MFR: g gallic acid equivalents in a bio-oil cut/g bio-oil in a bio-oil cut
2. For the 2-D MFR hot or cold condenser bio-oil cut: g gallic acid equivalents in a hot or cold condenser bio-oil cut/g bio-oil in a hot or cold condenser bio-oil cut

The antioxidant recovery for both the 1-D and 2-D MFR was defined as:

1. For the 1-D MFR bio-oil cut: g gallic acid equivalents in a bio-oil cut/g gallic acid equivalents in all bio-oil cuts
2. For the 2-D MFR hot or cold condenser bio-oil cut: g gallic acid equivalents in a hot or cold condenser bio-oil cut/g gallic acid equivalents in all bio-oil cuts of 1-D MFR

The antioxidant productivity for both the 1-D and 2-D MFR was defined as:

1. For the 1-D MFR bio-oil cut: g gallic acid equivalents in a bio-oil cut/100 g of pyrolyzed biomass
2. For the 2-D MFR hot or cold condenser bio-oil cut: g gallic acid equivalents in a hot or cold condenser bio-oil cut/100 g of pyrolyzed biomass
7.2.8  Char production

The char production was described in chapter 6. Briefly, the reactor temperature was increased at 10 °C/min from ambient to 565 °C with a holding time of 30 min when the reactor temperature reached 200, 300, 400 and 565 °C. We optimized the reactor operating conditions to produce the high yield of valuable chemicals in the bio-oil. The char was obtained as a by-product. More than 3 batches of char were collected from hydrolysis and organosolv lignin.

7.2.9  Activated carbon production through JBR

The production of activated carbon from hydrolysis and organosolv lignin char through a JBR was completed as described in chapter 6. Briefly, 10 g of char was fed into the JBR and activated from ambient to the selected temperature using the JBR. Carbon dioxide at the flow rate of approximately 190 mL/min was used to activate the char for 1 h in the reactor from ambient to 650, 700, 750 or 800 °C.

7.2.10 Optimization of the activated temperature to maximize MB adsorption

It was noted that the unwashed activated carbon/control (char) of hydrolysis and organosolv lignin did not adsorb MB, so the activated carbon/control (char) was washed with reverse osmosis (RO) water and the MB adsorption test was performed. The 0.5 g activated carbon/control (char) of hydrolysis and organosolv lignin was added to a 50 mL BD FALCON™ polypropylene conical tube (BD Biosciences, Bedford, MA, USA), 25 mL RO water was added and then the tube was mixed at 23 °C for 24 h using a wrist hand shaker (Wrist Action™ Shaker, Model 75, Burrell Scientific, Pittsburgh, PA, USA). Afterwards the water was drained and the wet char air dried for 24 h and dried again at 110 °C for 1 h. The dried char was then used in MB adsorption test. The optimization of the activation temperature was described in chapter 6. Briefly, 50 mg of each activated carbon sample was weighed and added to a 50 mL BD FALCON™ polypropylene conical tube and 25 mL of MB at the concentration of 500 mg/L was added to the
activated carbon sample. The sample was shaken at 23 °C for 24 h using a wrist hand shaker and centrifuged at 3000 r.p.m. (2056 r.c.f.) (Beckman GP Centrifuge, Beckman Coulter, Fullerton, CA, USA) for 10 min. The absorption was taken at 665 nm using a PowerWave™ XS microplate spectrophotometer (BioTek®, Winooski, VT, USA). The pH was determined for each sample after 24 h contact time.

7.2.11 Statistical analysis

Probit analysis (SAS version 9.2) was used to determine the LC50 values (mg/mL) and 95% fiducial limit (F.L.) of each of the bio-oil temperature cuts. Significant differences between LC50 values (mg/mL) were agreed on when 95% F.L. did not overlap.

7.3 Results

The hydrolysis and organosolv lignin produced the highest amount of raw bio-oil in the 300-400 °C reactor temperature range compared to the other bio-oil cuts (d.f.=7,8; F=102.35; P<0.0001) (Fig. 7.1) and it was approximately 59 and 64%, respectively of the total raw bio-oil yield. The hydrolysis and organosolv lignin produced the raw bio-oil yield of 47 and 40%, respectively for the ambient-565 °C reactor temperature range.

When dried, the bio-oil yield was 10% for both the hydrolysis and the organosolv lignin and the 300-400 °C bio-oil cut was 57% and 56%, respectively, of total dried bio-oil (d.f.=7,8; F=91.64; P<0.0001) (Fig. 7.1).
Fig. 7.1 Hydrolysis and organosolv lignin raw and dried bio-oil yield ± S.E. by 1-D MFR pyrolysis. Bars with the same lower case or upper case letter indicate no significant difference between the raw or dried bio-oil yield for the biomass across the reactor temperature cut (Two-way ANOVA, PROC GLM, Tukey’s HSD Test, P>0.05). raw: raw bio-oil; dried: dried bio-oil.
Table 7.1 The estimated 48 h LC$_{50}$ values (mg/mL) and 95% fiducial limit (F.L.) for Colorado potato beetle larvae exposed to different 1-D MFR hydrolysis and organosolv lignin bio-oil temperature cuts.

<table>
<thead>
<tr>
<th>Reactor temperature cuts (°C)</th>
<th>Concentration (mg dried bio-oil/mL solution)</th>
<th>LC$_{50}$ (95% F.L.) (mg dried bio-oil/mL solution)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hydrolysis Lignin</td>
<td>Organosolv Lignin</td>
</tr>
<tr>
<td>ambient-565$^a$</td>
<td>16-30</td>
<td>12-23</td>
</tr>
<tr>
<td>ambient-200</td>
<td>40-60</td>
<td>42-60</td>
</tr>
<tr>
<td>200-300</td>
<td>35-50</td>
<td>34-48</td>
</tr>
<tr>
<td>300-400</td>
<td>24-38</td>
<td>24-37</td>
</tr>
<tr>
<td>400-565</td>
<td>5-15</td>
<td>6-16</td>
</tr>
</tbody>
</table>

$^a$ Bio-oils produced from individual bio-oil cuts (ambient-200, 200-300, 300-400 and 400-565 °C) were then combined on the basis of their respective yields.

The median lethal toxicity (LC$_{50}$) was determined using at least five concentrations of each bio-oil (Table 7.1). The LC$_{50}$s determined with the CPB bioassay for the different 1-D MFR hydrolysis bio-oil cuts indicated that the toxicity of the 400-565 °C bio-oil cut was the highest, approximately 5-times greater than ambient-200 °C cut and 2-times greater than the ambient-565 °C combined cuts. The toxicity of the 1-D MFR organosolv 400-565 °C bio-oil cut was the highest, approximately 5-times greater than ambient-200 °C cut and 1.5-times greater than the ambient-565 °C cut. The probit model fit the experimental data well (P>0.05) when the analyses were done to obtain the toxicity (LC$_{50}$ values in mg/mL) of bio-oil cuts.

The 400-565 °C bio-oil cut of hydrolysis and organosolv lignin contained the highest amount of insecticidal chemicals compared to the other bio-oil cuts since the bio-oil cut was estimated to kill the highest number of CPB based on the yield and toxicity (Table 7.2). The method for determining the number of CPB killed using the bio-oil was described in chapter 4.
Table 7.2 The estimated toxicity of different 1-D MFR hydrolysis and organosolv lignin bio-oil cuts from 100 g biomass based on yield and median lethal concentration (LC$_{50}$).

<table>
<thead>
<tr>
<th>Reactor temperature cuts (°C)</th>
<th>Dried bio-oil (g)</th>
<th>LC$_{50}$ (mg/mL)</th>
<th>Number of CPB killed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hydrolysis lignin</td>
<td>organosolv lignin</td>
<td>hydrolysis lignin</td>
</tr>
<tr>
<td>ambient-565$^a$</td>
<td>10.343</td>
<td>9.452</td>
<td>21.6</td>
</tr>
<tr>
<td>ambient-200</td>
<td>0.065</td>
<td>0.084</td>
<td>48.9</td>
</tr>
<tr>
<td>200-300</td>
<td>1.380</td>
<td>0.808</td>
<td>43.1</td>
</tr>
<tr>
<td>300-400</td>
<td>5.858</td>
<td>5.248</td>
<td>30</td>
</tr>
<tr>
<td>400-565</td>
<td>3.041</td>
<td>3.313</td>
<td>9.9</td>
</tr>
</tbody>
</table>

$^a$ Bio-oils produced from individual bio-oil cuts (ambient-200, 200-300, 300-400 and 400-565 °C) were then combined on the basis of their respective yields.

Hydrolysis and organosolv lignin dried bio-oil at 0.3 mg/mL was also assessed for antimicrobial activity using (1) fungi (PU, RS and SS); and (2) bacteria (CM and XC). Among the four bio-oil cuts of hydrolysis lignin only the 400-565 °C bio-oil cut inhibited SS growth. The remainder of the bio-oil cuts had no inhibitory activity with the other fungi and bacteria (data not shown). Among the four bio-oil cuts of organosolv lignin, only the 400-565 °C bio-oil cut inhibited PU and SS growth. The other organosolv bio-oil cuts had no inhibitory activity with the other fungi and bacteria tested. Since the bio-oil cuts at low concentration (0.3 mg/mL) had little inhibitory effect on most of the selected fungi and bacteria tested, it was decided to proceed with only the insect bioassay for further screening of pesticidal activity. Furthermore, the CPB bioassay was easier to obtain results within a short period of time.

The 400-565 °C bio-oil cut was fractionated by using hot (120 and 150 °C) and cold temperature condensers (0 °C) in a 2-D MFR since the 400-565 °C bio-oil cut was determined to be the most toxic to CPB compared to the other bio-oil cuts. The yield of hydrolysis and organosolv lignin raw bio-oil collected in the 120 °C condenser was 4 and 5-times higher than that in corresponding cold condenser, respectively (d.f.=3.4; F=364.33; P<0.0001) (Fig. 7.2). For both the hydrolysis and organosolv lignin bio-oil
cuts the 120 °C condenser collected the higher amount of dried bio-oil than that in cold condenser (d.f.=3,4; F=353.51; P<0.0001). For both the hydrolysis and organosolv lignin bio-oil cuts the 150 °C condenser collected the higher amount of dried bio-oil than that in cold condenser (d.f.=3,4; F=214.67; P<0.0001).

![Graph showing bio-oil yield](image)

**Fig. 7.2** Hydrolysis and organosolv lignin bio-oil yield in different hot and cold condensers when the 2-D MFR reactor temperature is 400-565 °C. Bars with the same upper case or lower case letter indicate no significant difference between the raw or dried bio-oil yield for the biomass across the condenser temperature cut (Two-way ANOVA, PROC GLM, Tukey’s HSD Test, P>0.05). raw = raw bio-oil; dried = dried bio-oil.

The insecticidal activity was same for the bio-oil collected from the 150 °C and 120 °C condenser for both types of lignin produced with the reactor at 400-565 °C based on 95% F.L. (Table 7.3). The hydrolysis and organosolv lignin bio-oil cuts collected from the 150 °C condenser were not different in terms of the toxicity.
Table 7.3 The 48 h LC$_{50}$ values and 95% fiducial limit (F.L.) determined for hydrolysis and organosolv lignin bio-oil cuts produced with the 2-D MFR at 400-565 °C and collected in the 120 and 150 °C condensers using the CPB bioassay.

<table>
<thead>
<tr>
<th>Hot condenser temperature(°C)</th>
<th>LC$_{50}$ (95% F.L.)</th>
<th>(\text{mg dried bio-oil/mL solution})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hydrolysis lignin</td>
<td>Organosolv lignin</td>
</tr>
<tr>
<td>120</td>
<td>7.2 (6.0-9.0)</td>
<td>5.4 (3.5-6.7)</td>
</tr>
<tr>
<td>150</td>
<td>4.7 (2.0-6.0)</td>
<td>4.7 (3.1-6.7)</td>
</tr>
</tbody>
</table>

The 2-D MFR hydrolysis and organosolv lignin bio-oil cut of 400-565 °C collected in a 150 °C condenser (Table 7.4) killed approximately 1.7 and 1.8-times more CPB, respectively compared to 400-565 °C of 1-D MFR bio-oil cut (Table 7.2).

Table 7.4 The estimated toxicity of different 2-D MFR hydrolysis and organosolv lignin bio-oil cuts collected in 120 and 150 °C hot condenser based on yield and median lethal concentration (LC$_{50}$).

<table>
<thead>
<tr>
<th>Hot condenser temperature(°C)</th>
<th>Dried bio-oil (g)</th>
<th>LC$_{50}$ (mg/mL)</th>
<th>Number of CPB killed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hydrolysis lignin</td>
<td>organosolv lignin</td>
<td>hydrolysis lignin</td>
</tr>
<tr>
<td>120</td>
<td>2.84</td>
<td>3.18</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>6574</td>
<td>9815</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>2.42</td>
<td>2.87</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>8582</td>
<td>10177</td>
<td></td>
</tr>
</tbody>
</table>

The antioxidant activity in gallic acid equivalent concentration for the hydrolysis and organosolv lignin bio-oil produced between 300-400 °C by 1-D MFR pyrolysis was the highest compared to the other bio-oil cuts of the same biomass (\(\text{d.f.}=7,8; F=92.00; P<0.0001\)) (Fig. 7.3). There was no significant difference between the gallic acid equivalent concentration in the hydrolysis and organosolv lignin bio-oil for the same reactor temperature range (\(P>0.05\)) except for the 300-400 °C range.
Fig. 7.3 Gallic acid equivalent concentration ± S.E. from the hydrolysis and organosolv lignin bio-oil cuts produced at ambient-565 °C by 1-D MFR pyrolysis.

The antioxidant recoveries of hydrolysis and organosolv lignin biomass were 95 and 99%, respectively for the 1-D reactor temperature range of 300-400 °C (d.f.=7,8; F=621.69; P<0.0001) (Fig. 7.4). There was a significant difference between the antioxidant recovery of hydrolysis and organosolv lignin bio-oil at 300-400 °C reactor temperature range (P<0.05).
Fig. 7.4 Percent of total gallic acid equivalent recovered ± S.E. from the hydrolysis and organosolv lignin bio-oil cuts produced at ambient-565 °C by 1-D MFR pyrolysis.

Approximately 12 and 19% antioxidant productivity in total were produced from hydrolysis and organosolv lignin bio-oil cuts, respectively, through 1-D MFR when 100 g biomass was pyrolyzed (Fig. 7.5). There was a significant difference between the antioxidant productivity of hydrolysis and organosolv lignin biomass at 300-400 °C reactor temperature range (d.f.=7,8; F=366.92; P<0.0001).
Fig. 7.5 Gallic acid equivalent ± S.E. determined for the different hydrolysis and organosolv lignin bio-oil cuts produced from 100 g biomass through 1-D MFR.

The 300-400 °C bio-oil cut was fractionated by using the hot/cold condenser combinations of 120/0 °C and 150/0 °C with the 2-D MFR. The hydrolysis and organosolv lignin bio-oil collected in the 120 °C hot condensers was 3 and 6-times greater compared to the corresponding cold condenser for the reactor temperature range of 300-400 °C (d.f.=3,4; F=204.71; P<0.0001) (Fig. 7.6). The hydrolysis and organosolv lignin bio-oil yield collected in the 150 °C hot condensers was 2 and 4-times greater compared to the corresponding cold condensers (d.f.=3,4; F=93.47; P<0.05) (Fig. 7.6).
Fig. 7.6 Hydrolysis and organosolv lignin bio-oil yield ± S.E. collected with the two hot/cold condenser combinations (120/0 °C and 150/0 °C) with the 2-D MFR reactor temperature range of 300-400 °C.

The gallic acid equivalent concentration in the organosolv lignin bio-oil collected in the 150 °C condenser was higher compared to the 120 °C condenser temperature (d.f.=3,4; F=62.80; P=0.0008) (Fig. 7.7). Similarly, the gallic acid equivalent concentration in organosolv was higher in both hot temperature condensers than compared to the concentration measured in the corresponding hydrolysis lignin bio-oil collections (P<0.05). The g gallic acid equivalents/g bio-oil in the 0 °C condenser for each hot/cold condenser combination was excluded from the graph since these were consistently less than 15%.
Fig. 7.7 Gallic acid equivalent concentration of hydrolysis and organosolv lignin bio-oil cuts produced from 120 and 150 °C hot condenser with the 2-D MFR reactor temperature range of 300-400 °C.

The percent gallic acid equivalents recovered in the 0 °C condenser for each hot/cold condenser combination for the hydrolysis and organosolv lignin bio-oil was excluded from the graph since these were consistently less than 3% when the reactor temperature range was 300-400 °C (Fig. 7.8). The highest antioxidant recovery for the organosolv lignin bio-oil was in the 150 °C condenser, with 96% recovered, from the total 1-D MFR bio-oil cuts (d.f.=3.4; F=40.68; P=0.0019) (Fig. 7.8).
Fig. 7.8 Percent of total gallic acid equivalent recovered ± S.E. from the hydrolysis and organosolv lignin bio-oil cuts collected in the 120 and 150 °C hot condensers with the 2-D MFR reactor temperature range of 300-400 °C.

The antioxidant productivity was greater in the 150 °C condenser organosolv lignin bio-oil compared to any other hot condenser bio-oil for each 100 g of hydrolysis and organosolv lignin biomass pyrolyzed (d.f.=3.4; F=197.78; P<0.0001) (Fig. 7.9).
Fig. 7.9 Gallic acid equivalent ± S.E. from the hydrolysis and organosolv lignin bio-oil cuts collected in the 120 and 150 °C hot condensers between 300-400 °C reactor temperature range by 2-D MFR produced from 100 g biomass.

The hydrolysis and organosolv lignin activated carbon yield decreased with activation temperature (Fig. 7.10) with highest yield of organosolv lignin activated carbon produced at 650 °C.
Fig. 7.10 Percent yield of hydrolysis and organosolv lignin activated carbon ± S.E. at four different activation temperatures.

The hydrolysis and organosolv lignin activated carbon and the respective char produced no MB adsorption at 24 h contact time, even after the water washing pre-treatment (Fig. 7.11). After 24 h the concentration of MB in the solution was higher than that of in the initial concentration. The pH for all samples including the char after 24 h contact time was within the range of 8.9-9.2. It was not possible to study the Langmuir or Freundlich isotherm since there was no measurable MB adsorption with either the hydrolysis or organosolv lignin activated carbon.
Fig. 7.11 Average methylene blue adsorption (mg methylene blue adsorbed/g char or activated carbon) ± S.E. in a 24 h contact time by char and hydrolysis and organosolv lignin activated carbon produced at four different activation temperatures.

7.4 Discussion

The pyrolysis of hydrolysis and organosolv lignin by the 1-D MFR produced 47 and 40% raw bio-oil, respectively, compared to the organosolv lignin raw bio-oil yield of 34% produced by 1-D MFR in my earlier work [Hossain 2011]. The MFR reactor size and operating conditions affect the bio-oil yield. In this present study the reactor was 15 cm in diameter and 25.4 cm in height. The biomass was pyrolyzed from ambient to 565 °C and the temperature was held for 30 min at the end of each cut. In my previous study [Hossain 2011] the reactor was 8.8 cm in diameter and 11.5 cm in height. The biomass was pyrolyzed from ambient to 600 °C and the temperature was held for 5 min at the end of each cut. As the MFR size and the operating conditions are different the vapor residence time will be different also for the same biomass although in both studies we did not study the vapor residence time.
The MFR and traditional fluidized bed reactor work different way to produce the biomass bio-oil and as a result the bio-oil yield is not same. The organosolv lignin bio-oil yield by MFR was higher compared to the same biomass bio-oil yield (20%) produced through a traditional fluidized bed reactor [Hossain et al. 2013]. In my previous study [Hossain 2013] the volume of fluidized bed reactor was different than that of MFR of present study. The biomass was pyrolyzed at 550 °C. Nitrogen and silica sand were used as the fluidizing gas and the bed material, respectively. The traditional fluidized bed reactor and MFR perform the pyrolysis in different way. As a result the vapor residence time is not same for the two types of reactor. It is speculated that there is more contact time between vapor and char in MFR compared to that in fluidized bed reactor since we do not introduce any inert gas to fluidize the biomass. In addition, there is no bed material in MFR. No previous studies have examined hydrolysis lignin bio-oil produced by either the fluidized bed or MFR reactor.

In this study the 1-D and 2-D MFR reactor temperature was optimized to concentrate and recover the lignin bio-oil fractions with the highest antioxidant and pesticidal activity. Most of the antioxidant and pesticidal activity was measured between the 300-400 °C and 400-565 °C reactor temperature range, respectively. The LC50 of the organosolv lignin 1-D MFR combined bio-oil cut (ambient-565 °C) was approximately 1.5-times higher than that of hydrolysis lignin 1-D MFR combined bio-oil cut (ambient-565 °C) when the dried bio-oil yield was approximately 1% less. The organosolv lignin is pure lignin whereas the hydrolysis lignin contains only 55-57% lignin. This result supports the previous study which found the CPB toxicity of lignin bio-oil was stronger than that of the mixture of lignin, cellulose and hemicellulose biomass bio-oil [Hossain et al. 2013].

It was demonstrated previously that nicotine could be concentrated in the bio-oil fraction when the condenser temperature was optimized using the 2-D MFR [chapter 3]. The 2-D MFR hydrolysis and organosolv lignin 150 °C produced more toxic compounds compared to 120 °C hot condenser bio-oil. The toxicity of the 150 °C condenser bio-oil doubled for both types of lignin pyrolyzed by 2-D MFR compared to the bio-oil produced by the 1-D MFR, when the dried bio-oil yield was reduced by 20 and 13% for hydrolysis and organosolv lignin, respectively. It is speculated that some high volatiles might have
antagonistic effect with the low volatiles when the bio-oil was collected in cold condenser of 1-D MFR [Hossain et al. 2013].

The LC$_{50}$ of the most active bio-oil fraction collected in the electrostatic precipitator (ESP) (an ESP is a filtering device to remove fine particles, like dust from the flowing gas using an electrostatic charge) after pyrolysis of organosolv lignin in the bubbling bed reactor was 5.1 mg/mL [Hossain et al. 2013]. The ESP dried bio-oil yield was 4.7%, approximately 2-times higher than the yield collected from the 150 °C condenser after the pyrolysis of both hydrolysis or organosolv lignin by 2-D MFR between 400-565 °C. However, the ESP organosolv lignin bio-oil CPB bioassay toxicity was slightly lower than the MFR organosolv lignin bio-oil, indicating the latter MFR 2-D process may still concentrate the active compounds to a greater degree than the bubbling bed reactor.

Another limitation noticed during the separation of pesticidal compounds after pyrolysis of biomass in the fluidized bed reactor was the higher amount of contaminants produced, for example PAHs that are not desirable in the bio-oil [Hossain et al. 2015]. In addition, it is necessary to separate the active compounds from the larger amount of water present in the bubbling bed bio-oil [Hossain et al. 2013 and 2015]. This is an additional expense required to purify the pesticidal compounds available in the fluidized bed reactor bio-oil that is not necessary after 2-D MFR bio-oil production. The organosolv lignin organic phase bio-oil cut at 250-300 °C 1-D MFR reactor temperature range produced the highest CPB mortality compared to the other bio-oil cuts [Hossain 2011], but since the LC$_{50}$ (mg/mL) was not determined in that study it is difficult to make a direct comparison.

The antioxidant activity in the bio-oil produced from hydrolysis and organosolv lignin and collected after 300-400 °C heating in the 1-D MFR was estimated to be 11 and 19% recovery of the total bio-oil produced in all cuts combined, respectively, while the antioxidant concentration in that bio-oil cut was measured at 41 and 74%, respectively. The lignin purity in the hydrolysis and organosolv lignin feedstock was approximately 50 and 100%, respectively; therefore, it is reasonable that the organosolv lignin produced 8% more antioxidant than that of hydrolysis lignin as it has been noted previously that phenolic compounds and antioxidant activity are proportional to the amount of lignin [Kurakake et al. 2015]. It was also observed in this study that the 150 °C condenser on
the 2-D MFR was responsible for a higher recovery and concentration of antioxidant activity from the organosolv lignin bio-oil than from the hydrolysis lignin bio-oil under the same conditions. Although, the 2-D MFR organosolv lignin bio-oil had 3% less recovery, it had an 18% greater concentration of antioxidant activity compared to the same reactor temperature bio-oil cut produced from the 1-D MFR. There is a possibility that the some low volatiles decrease the antioxidant activity of high volatiles when the bio-oil was collected in a cold condenser of 1-D MFR. In contrast, the 2-D MFR hydrolysis lignin produced lower concentrated and lower recovery of antioxidants in the hot condenser bio-oil compared to that of 1-D MFR condenser bio-oil for the 300-400 °C reactor temperature range. The impurity of hydrolysis lignin might be the main reason for that result since some antioxidant compounds in hydrolysis lignin different from those in organosolv lignin might degrade after reheating the produced vapor in a hot condenser of 2-D MFR reactor [Gálico et al. 2015]. Another possibility the mixture of low and high volatiles of hydrolysis lignin 1-D MFR 300-400 °C bio-oil cut produces high antioxidant activity whereas 2-D MFR 300-400 °C hot condenser bio-oil cut with low volatiles produces low antioxidant activity. Prior to pyrolysis biomass is often treated to separate different components, for example cellulose and lignin during the cellulosic ethanol production steps. However, this pre-treatment can have an effect on the antioxidant activity of the resulting lignin bio-oil. Untreated Akamatsu (Pinus densiflora) had higher antioxidative activity compared to the same biomass degraded by ammonia water treatment at high temperature although new antioxidants were produced from the lignin degraded by the treatment [Kurakake et al. 2015]. This suggests that the type of biomass pre-treatment will also affect the thermostability of the resulting antioxidants.

In a comparison of which type of lignin is better for pesticide and antioxidant production, it can be observed that both have positive and negative qualities. Both hydrolysis and organosolv lignin are produced as a by-product in the cellulosic ethanol industries, but the pre-treatment of organosolv with organic solvents is a more expensive, both from a cost and environmental perspective. However, the solvent treatment increases the enzymatic digestibility of the biomass and as a result ethanol production increases [Mesa et al. 2016, Teramoto et al. 2008], and the organosolv lignin produces bio-oil with greater
antioxidant activity compared to hydrolysis lignin. In contrast, less ethanol is produced during hydrolysis lignin production since some cellulose is unconverted in this process. However, since the hydrolysis lignin production does not require organic solvent [Kuglarz et al. 2016], this process is comparatively less harmful for the environment.

Water-washed hydrolysis and organosolv lignin activated carbon produced at four different activation temperatures did not adsorb MB from the solution. We performed the adsorption test at pH approximately 9. The pH of the solution may enhance or suppress MB uptake by altering the adsorbent surface chemistry [Qada et al. 2006]. With gradually increased pH, the uptake amount of MB onto activated carbon produced from NaOH-pretreated rice husks increased from 202.3 mg/g to 389.9 mg/g [Lin et al. 2013]. Another possibility some compounds were extracted from activated carbon and reacted with MB to produce high light absorption. Colomba [2015] produced activated carbon from kraft lignin using a JBR. The kraft lignin was heated to the pyrolysis temperature of 475 to 550 °C in the JBR, and the resulting char was activated between 800 to 900 °C. The adsorption capacity for the kraft lignin was approximately 100 mg MB/g activated carbon depending on the mesopore volume of activated carbon (cm³/g). That study did not produce activated carbon using the hydrolysis and organosolv lignin as a feedstock.

7.5 Conclusions

A high concentration of compounds with pesticidal and antioxidant activity was produced in different bio-oil cuts during the pyrolysis of hydrolysis and organosolv lignin using a 2-D MFR at the optimal reactor and condenser temperatures. All the bio-oil cuts from lignin biomass contain valuable compounds. The ambient-300 and 400-565 °C bio-oil cuts have insecticidal effects whereas the 300-400°C bio-oil cut has antioxidant activity. A biorefinery based on the 2-D MFR is a promising process for isolating high value products from lignin. Further study is required to identify the chemical compounds in the 2-D MFR active lignin bio-oil cuts using gas chromatography and mass spectrometry. More tests are required to optimize and control the pH of dye solution during the contact time for MB adsorption onto the hydrolysis and organosolv lignin activated carbon.
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Chapter 8

8 General conclusions and recommendations

8.1 General conclusions

The objective of my project was to recover the valuable chemicals from agricultural waste through pyrolysis. Nicotine was the model compound for my experiments and was chosen for this study because it is thermostable under pyrolysis conditions [Booker et al. 2010] and since it has promising medicinal applications [Jin et al. 2013]. I optimized the reactor operating conditions to increase nicotine recovery from tobacco leaves and showed that total nicotine recovery from 1-D MFR pyrolysis was higher than the conventional single solvent extraction of biomass. The results indicate that it is an efficient method of nicotine recovery and it is environmentally friendly since it does not need solvents. The nicotine concentration in the bio-oil was also increased by 8% although the nicotine recovery was decreased approximately 20% through a drying process. The 2-D MFR process improved the latter problem by separating the water and other high volatiles using a hot temperature and an ice bath condenser system. The 2-D MFR pyrolysis process allowed for a total nicotine recovery of approximately 90% and a bio-oil nicotine concentration of 20%.

The concept of chemical recovery by 2-D MFR was also applied to produce a higher concentration of pesticidal and antioxidant compounds from tobacco, tomato, spent coffee, hydrolysis lignin and organosolv lignin biomass bio-oils. The tobacco, tomato, hydrolysis lignin and organosolv lignin 2-D MFR bio-oil showed a promising increase in pesticidal activity and the antioxidant concentration was increased for the tobacco, tomato and organosolv lignin by the 2-D MFR compared to 1-D MFR bio-oil. I was also able to demonstrate that the adsorption capacity of tomato activated carbon was better than the commercial coconut shell activated carbon even though the tomato char was not pretreated before carbonization. Of the different biomass I pyrolyzed, the tomato plant
residue provided the greatest number of valuable co-products (pesticidal compounds, antioxidants and activated carbon) available for further application.

My findings are an example of the many chemicals that can be obtained from a biorefinery where phytochemicals can be recovered from the plant waste, for example 160 compounds identified from *Populus* (poplar) tree species [Devappa et al. 2015]. Potential phytochemical co-products can be obtained from the staple crops [Ding and Xu 2014]. The starch, non-starch polysaccharides, protein and fat could be fractionated from flex [Zijlstra et al. 2012]. During bioethanol production from corn dry milling process, flavonoids and other chemicals are produced as co-products [Rausch and Belyea 2006] as well as bio-oil and syn-gas from lignocellulose biomass [Ding and Xu 2014].

In summary, from the biorefinery standpoint the MFR provides a full range of valuable products, especially when tomato plant residue is pyrolyzed.

### 8.2 Recommendations

The findings of this thesis indicate that the mechanically fluidized reactor, both the one- and two-dimensional versions, has an important function in a thermochemical biorefinery process. There are of course several improvements that should be made to further optimize the operation of the MFR as well as its condensation train. Recommendations to improve the recovery of valuable chemicals and the quality of the final products are as follows:

1) Narrow down the 2-D MFR reactor temperature cuts and for each reactor temperature cut use more than two condensers in series at different temperatures depending on the target compounds concentration and recovery in the bio-oil.

2) Each bio-oil cut obtained from the 2D MFR process does not have as many compounds as Booker et al. [2010] identified in the raw bio-oil. The solvent extraction procedures developed by Booker et al. [2010] could be applied to the cuts from the 2D MFR to obtain mixtures with a much smaller number of components. A sophisticated liquid chromatography-mass spectrometry (LC-MS) equipment such as the Orbitrap
might then be applied to detect the compounds that are not volatile and from which GC-MS is not suitable.

3) When biomass including lignin [Shen et al. 2015] is pyrolyzed, the bio-oil cuts from the 2D MFR that contain phenolic compounds could be used to substitute for phenol produced from non-renewable resources in the production of phenol-formaldehyde resins [Choi et al. 2015].

4) The 2D MFR process could be used to obtain other thermo-stable compounds that have promising properties. For example, Paclitaxel (Taxol®) has an application for cancer treatment [Barbuti and Chen 2015] and is available in bark extract of the Pacific yew tree, Taxus brevifolia. The grape seed extract contains proanthocyanidins that have antioxidant and anti-inflammatory properties [Chu et al. 2016]. The Pacific yew tree bark and grape seed are readily available in Canada and could be pyrolyzed to produce paclitaxel and proanthocyanidins, respectively.

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