Microwave-assisted alkali pretreatment, densification and enzymatic hydrolysis of canola straw and oat hull

A Thesis Submitted to the

College of Graduate and Postdoctoral Studies in
Partial Fulfillment of the Requirements for the Degree of
Master of Science (M.Sc.)

In the
Department of Chemical and Biological Engineering
University of Saskatchewan

By

Obiora Samuel Agu

Permission to Use

In presenting this thesis in partial fulfillment of the requirements for a Master of Science from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission to copy this thesis in any manner, in whole or in part, for scholarly purposes may be granted by Dr. Lope Tabil who supervised my thesis work or, in his absence, by the Head of the Department or the Dean of the College of Engineering. It is understood that any copying, publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in a scholarly use which may be made of the material in my thesis. Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

<div align="center">

Head of the Department of Chemical and Biological Engineering

University of Saskatchewan

57 Campus Drive

Saskatoon, Saskatchewan

Canada S7N 5A9

</div>

Abstract

Production of bioethanol from lignocellulosic biomass is very challenging due to the complex nature of the feedstocks. Pretreatment is a necessary step for efficient and effective conversion of lignocellulosic biomass to biofuel. This study investigated the effects of microwave-assisted alkali pretreatment, microwave pretreatment (distilled water) and untreated canola straw and oat hull on the pellet quality, and the conversion of microwave pretreated and alkali treated substrates into sugar for cellulosic bioethanol production. Microwave pretreatments were carried out by immersing the biomass in dilute alkali solutions (NaOH and KOH) at various concentrations of 0, 0.75 and 1.5% (w/v) for microwave-assisted times of 6, 12, and 18 min, and at a fixed microwave power of 713 W. Alkali treatments were carried out by soaking and the same alkali used for the microwave pretreatment. The biomass and alkali concentrations remained unchanged.

Chemical composition analysis of canola straw and oat hull showed that the application of microwave-assisted alkali pretreatment on the biomass disrupted and broke down the lignocellulosic structure of canola straw and oat hull compared to microwave pretreatment and alkali treatment. Images acquired using a scanning electron microscope (SEM) revealed the structural changes caused by microwave-assisted alkali pretreatment on biomass samples.

Pellet characteristics such as density, tensile strength and dimensional stability were used to describe the pellets produced from single pelleting technique. The results showed that the interaction effects of alkali concentration and microwave heating time significantly affected the physical characteristics of canola straw and oat hull pellets.

Upon enzymatic saccharification, microwave-assisted alkali pretreatment and alkali treatment of canola straw and oat hull on enzymatic saccharification were investigated. Microwave-assisted alkali pretreatment showed the highest glucose yield in treatment combinations of 1.5% NaOH/18 min for canola straw and 0.75% NaOH/18 min for oat hull sample ground in a1.6 mm hammer mill screen size. Overall, microwave/NaOH pretreatment resulted in better physical quality pellets from both canola straw and oat hull samples than microwave/KOH pretreatment. Also, microwave pretreatment and alkali treatment using NaOH solution at different concentrations resulted in higher glucose yields compared to KOH in both feedstocks.

Acknowledgements

I would like to express my gratitude to my supervisor Professor Lope G. Tabil for his invaluable encouragement and guidance during my research program. He was a pleasure to work with and was a source of inspiration. He has been very understanding and being flexible with my academic and research works since I started my graduate studies in College of Engineering. I would also like to appreciate all the members of my Graduate Advisory Committee Professors Oon-Doo Baik, Venkatesh Meda and Dr. Tim Dumonceaux for providing me with constructive criticism during the course of my research work.

I am very grateful to Dr. Tim Dumonceaux of Agricultural and Agri-Food Canada – Saskatoon Research Centre (AAFC-SRC) for allowing me to use their laboratories and facilities.

Technical support extended during my experiments by Louis Roth of Chemical and Biological Engineering Department and Nan Fang Zhao of Mechanical Engineering Department, University of Saskatchewan; Dr. Jennifer Town of AAFC-SRC; Dr. Kingsley L. Iroba and Majid Soleimani of Chemical and Biological Engineering Department, University of Saskatchewan is highly appreciated. Also, I acknowledge the support of my office mates and colleagues: Charley Sprenger, Chidimma Afam Mbah, Robbarts Nongmaithem and Jim Funk.

The financial support received from the Tertiary Education Trust Fund (TETFund) through Enugu State University of Science and Technology Enugu, Nigeria and BioFuelNet Canada to successfully undertake the project is acknowledged.

I am also grateful to my parents, Mr. Dennis Emeka Agu and Mrs. Cordella Ifyinwa Agu for fulfilling their promise to God about my existence, for providing me with basic education and moral values towards the development of my researcher career. Also, I would like to thank my brother and sister: Onyekachukwu, Obinaujunwa and in-laws for their motivation, support and encouragement.

Most of all, I would like to thank my lovely wife Onyinyechukwu Nneka and children: Olilanyachukwu, Chukwudi and chukwudubem for their lovely and unwavering support during the course of my research work. They have been the source of my motivation and perseverance.

Most importantly, I dedicate everything to you Immaculate Conception, Blessed Virgin Mary, Mother of my LORD JESUS CHRIST for successfully completing my M.Sc. program. JESUS I trust in you.

Table of Contents

List of Tables

List of Figures

Chapter 1

1. Introduction and Objectives

1.1 Introduction

Lignocellulosic biomass production is attracting global interest because it is carbon neutral and a sustainable resource for industrial-scale energy production (Tumuluru et al. 2014). Lignocellulosic biomass is derived from agricultural and forest residues as well as industrial and municipal solid wastes. When converted to renewable energy, they are environmentally friendly with neutral carbon footprint compared to fossil energy sources such as crude oil, coal, and natural gas (Iroba 2014; Sarkar et al. 2012). The creation of renewable energy is a growing global concern as fossil fuel supplies run out and an energy crisis may arise (Perruzza 2010). Increasing population and industrial prosperity demands for energy contribute to excessive fossil fuel combustion particularly in urban areas. This has continued to increase environmental pollution via the emission greenhouse gas (Sarkar et al. 2012; Liu et al. 2014). These emissions result in acid rain which can damage the ecosystem and adversely affect human health (Demirbas 2004). Therefore, there is a need to replace the existing energy source with more reliable, renewable and environmentally friendly sources. According to Naik et al. (2010), the carbon dioxide released at fuel combustion is equivalent to the amount of carbon dioxide fixed by the plant due to the photosynthetic process and thus, the reduction of carbon dioxide via bioethanol is huge compared to fossil fuels.

In Canada, canola and oat are major crops grown in the prairies. Canola (*Brassica napus*), an oilseed, has estimated crop production as 15,555.1 Mega gram (Mg) and Saskatchewan production is 40% estimated at 8.9 Mg. The production of canola rose from 18.38 Mg in 2016 to 18. 5 Mg in 2017 and ranks second in world production behind European Union. No-tillage operation is being applied by about half of the canola growers in the Black soil zone of Saskatchewan. Oat (*Avena sativa L.*) crop production globally is estimated 2,907.5 Mg, making Canada one of the major supplier of oat in world trade. Saskatchewan 1.6 Mg, Manitoba and Alberta are the major producers in Canada. Oat variety depends on its usage. There are four main uses of oat such as livestock feed, human consumption, recreation horse feed and forage. The tillage operations are considered based on the varieties and where the oats are grown (Sask.

Seed Statistics Canada 2014; USDA FAS World Agricultural Production 2017; Sask. Govt. Oat Production and Markets Factsheet 2017). Annual production of agricultural crop residues in Canada calculated for over a period of ten years (2001 to 2010) is 82.4 million dry Mg (Li et al. 2012). Liu et al. (2014) reported that a total production of agricultural biomass was estimated to 37.3 (Mg) which was dominated by crop residues. Agricultural crop residues are potential biomass that can be used for sustainable production of bioethanol and biofuel (Adapa et al. 2009) and access is virtually unlimited abundant at low cost and readily available (Gong et al. 2010; Demirbas et al. 2009; Liu et al. 2005). Lignocellulosic biomass (second generation feedstock) are categorized in six groups: crop residues (corn stover, wheat straw, rice straw, barley straw, rice hulls, sweet sorghum bagasse), hardwood (aspen, poplar), softwood (pine, spruce), cellulose wastes (paper waste, recycled paper sludge), herbaceous biomass (alfalfa hay, switchgrass, bermudagrass, thimothy, miscanthus), and municipal solid waste (Sanchez and Cardona 2008). Agricultural and oilseed straws recently have been considered important feedstocks for bioenergy applications because of their low nutritional value when used as feed for animals (Tumuluru et al. 2014). Of the agricultural crop residues, straws are considered the most attractive feedstock for bioethanol production due to its high cellulose and hemicellulose contents which readily hydrolyze into fermentable sugars (Ibrahim 2012). These second generation feedstocks are readily available in many countries and reduce the environmental risks such as soil degradation and water and air pollution associated with first generation feedstock (grain and sugarcane) and has no competition with food crops (Smith 2013; Iroba 2014; Naik et al. 2010).

Also, agricultural crop residues in natural form are high in moisture content, loose and bulky, irregular shape and size, and difficult to handle, transport and store (Adapa et al. 2009; Tumuluru et al. 2014). Densification of the biomass is an efficient process in solving some of these engineering challenges (Tumuluru et al. 2014; Sokhansanj et al. 2005). Densification increases the bulk density of biomass from $40 - 200$ kg/m^3 to a final compact density of $600 - 1200$ kg/m^3 (Adapa et al. 2009; Kashaninejad and Tabil 2011; Mani et al. 2006) for efficient transport and storage, and at low moisture content (8% w.b) for safe storage (Mani et al. 2006). Iroba et al. (2014) reported that feedstocks pretreated with 1% w/v NaOH concentration results in higher density than untreated feedstocks. This is because pretreated feedstock release binding agent (lignin) which increase the adhesion within the particles, activate the intermolecular bonds

within the contact area of the samples and in addition improve the mechanical interlocking of the particles during pelletizing.

Bioethanol, C_2H_5OH, is a liquid fuel from plant materials such as sugar-based, corn-based and lignocellulosic agricultural wastes and crop residues (Gong et al. 2010; Demirbas et al. 2009; Tong et al. 2012; Perruzza 2010). The conversion of lignocellulosic bioethanol process is conducted in four steps: pretreatment, enzymatic saccharification, fermentation and product (ethanol) recovery (Quintero et al. 2011; van Zessen et al. 2003). Bioethanol from sugarcane was first introduced in Brazil to overcome the energy crisis in 1975 (Rosillo-Calle and Cortez 1998) and in 2006, it became the largest producers of bioethanol (18 billion liters) more than United States (Hettinga et al. 2008). In Canada, ethanol blend has facilitated positive ethanol-petrol mixtures (Ohgren et al. 2007). Five percent renewable content in gasoline and 2% renewable content in diesel fuel and heating oil have been implemented using ethanol blends in these products (Liu et al. 2014; Sorda et al. 2012). In the U.S., a 10% of total gasoline consumption by 2020 have been targeted corresponding to the production of 136 billion liters of biofuel using lignocellulosic biomass (Liu et. 2014).The lignocellulosic ethanol production has shifted from first generation feedstocks (grains and oilseeds) to second (cellulosic biomass from crop residues and dedicated energy crops) and third generation feedstocks (microalgae) in order to increase biofuel production (Smith 2013; Liu et al. 2014). First-generation ethanol has an average GHG emissions of 62 g and 56 g CO_2 eq.MJ^{-1} for wheat-grain and sugar beet respectively, while second generation ethanol emissions range from 24-30 g CO_2 eq.MJ^{-1} for woody crops and wheat straw (Whitaker et al. 2010). In addition, Whitaker et al. (2010) reported that the energy requirements for first-generation ethanol are much higher with wheat and sugar beet both at 0.7 MJ_{in}/MJfuel compared with second-generation ethanol which is at a range of 0.1–0.35 MJ_{in}/MJfuel. Overall, cellulosic ethanol could replace gasoline due to its reduction in GHG emission by 85% (Perruzza 2010).

The lignocellulosic biomass comprises cellulose, hemicellulose and lignin. These components are linked with the bioethanol conversion through preprocessing step called pretreatment (Karimi et al. 2013; Kurmar et al. 2009). The pretreatment of the biomass assists in cell wall breakdown, creating access for cellulose and hemicellulose to be hydrolyzed into fermentable sugars for bioethanol production. The different pretreatments methods involved are alkali and microwave-assisted pretreatment, dilute acid, steam explosion, ammonia fiber

explosion (AFEX), lime treatment and organic solvent treatments (Quintero et al. 2011; van Zessen et al. 2003; Mosier et al. 2005).

1.2 Research Objectives

The main objective of this research study is to investigate the effect of microwave-assisted alkali pretreatment of canola straw and oat hull and the subsequent densification on their enzymatic digestibility for conversion into bioethanol.

The following are the specific objectives that have been formulated for this research work:
1. to evaluate the effect of microwave-assisted alkali pretreatment in the disintegration and disruption of lignocellulosic biomass (canola straw and oat hull);
2. to densify the microwave-assisted alkali pretreated biomass and evaluate its pelletability, and other physic-chemical properties; and
3. to determine the extent of digestibility of cellulose and hemicellulose during enzymatic saccharification of pretreated and untreated biomass.

1.3 Organization of the Thesis

The thesis is organized according to the University of Saskatchewan for manuscript based thesis. This implies that the information, experimental data and analysis reported in this thesis have been published or in-press in peer-reviewed journals. The manuscripts were written and submitted for publication before the M.Sc. project was completed. The part of manuscript presented in Chapters 3 and 4 have been presented and accepted in a conference proceeding paper, and submitted to a peer-reviewed journal. Chapters 2, 3 and 4 are original text of this thesis included to introduce the subject matter and discuss the outcome of the project. The general discussion, conclusions and recommendations of the project are presented in Chapters 5 and 6.

1.4 Manuscript Content of the Thesis

The current M.Sc. research program has resulted in the contribution to knowledge in the densification and pretreatment of canola straw and oat hull leading for cellulosic ethanol production. In Chapter 3, the experiments on particle size reduction were performed on the

canola straw and oat hull using hammer mill. The effects of alkali concentration and microwave heating on the microwave-assisted alkali pretreated and microwave alone pretreated were investigated. The physical characteristics of the ground samples; ash content, bulk and particle densities for the microwave-assisted alkali pretreatment were measured. The data generated can be used in the design of a pilot-scale microwave-assisted alkali biomass pretreatment plant for bioethanol production. Also in Chapter 3, an experiment to study the engineering problems associated to particle handling, transportation and storage relating to tensile strength, dimensional stability and pellet density of densified canola straw and oat hull were investigated. The effect of the microwave-assisted alkali pretreatment was evaluated through chemical composition analysis of microwave-assisted alkali pretreated and microwave alone pretreated samples using National Renewable Energy Laboratory (NREL) standard. This method could be used by pellet industries to optimize the bioenergy production through thermochemical process. In Chapter 4, the optimum yield of cellulose from the chemical composition analysis results of microwave-assisted alkali, microwave alone, and alkali pretreated were selected for enzymatic saccharification with a combination of cellulase and β-glucosidase enzymes mixed together. The digested glucose in the sample was analyzed using dinitrosalicylic (DNS) acid method. This method can be adopted and applied by the bioethanol manufactures in estimating the accessible and digestible glucose (sugars) quantity after enzymatic saccharification. Furthermore, the extent of structural cell walls changes as a result of microwave-assisted alkali pretreatment, microwave alone pretreatment and alkali pretreatment were examined, and compared using scanning electron microscopy (SEM).

1.5 References

1. Adapa, P., L. Tabil, G. Schoenau. 2009. Compaction characteristics of barley, canola, oat and wheat straw. *Biosystems Engineering* 104: 335 – 344.

2. Demirbas, A., M.F.M. Balat and H. Balat. 2009. Potential contribution of biomass to the sustainable energy development. *Energy Conversion and Management* 50:1746-1760.

3. Demirbas, A. 2004. 'Bioenergy, global warming, and environmental impacts, energy sources, Part A: recovery. *Utilization and Environmental Effects* 26(3): 225 – 236.

4. Gong, G., D. Liu and Y. Huang. 2010. Microwave-assisted organic acid pretreatment for enzymatic hydrolysis of rice straw. *Biosystems Engineering* 107: 67 – 73.

5. Hettinga, W.G., H.M. Junginger, S.C. Dekker, M. Hoogwijk, A.J. McAloon and K.B. Hicks. 2008. Understanding the reductions in US corn ethanol production costs: an experience curve approach. *Energy Policy* 37(1):190-203.

6. Ibrahim, H. A. 2012. Pretreatment of straw for bioethanol production. *Energy Procedia* 14:542 – 551.

7. Iroba, K.L., L.G. Tabil, S. Sokhansanj, S and V. Meda. 2014. Producing durable pellets from barley straw subjected to radio frequency-alkaline and steam explosion pretreatments. *International Journal of Agricultural and Biological Eng*ineering 7(3): 68 - 82.

8. Iroba, K.L. 2014. Effect of pretreatment on breakdown of lignocellulosic matrix in barley straw as feedstock for biofuel production. Ph.D. thesis. Saskatoon, Saskatchewan: Department of Chemical and Biological Engineering, University of Saskatchewan.

9. Karimi, K., M. Shafiei and R. Kumar. 2013. Progress in physical and chemical pretreatment of lignocellulosic biomass. In *Biofuels Technologies,* ed. V.K. Gupta and M.G. Tuohy, 53 – 96: Verlag Berlin Heidelberg: Springer.

10. Kashaninejad, M. and L. G. Tabil. 2011. Effect of microwave: chemical pretreatment on compression characteristics of biomass grinds. *Biosystems Engineering* 108: 36 – 45.

11. Kumar, P., D. M. Barrett, M. J. Delwiche and P. Stroeve. 2009. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial and Engineering Chemistry Research* 48: 3713 – 3729.

12. Li, X., E. Mupondwa, S. Panigrahi, L.G. Tabil, S. Sokhansanj and M. Stumborg. 2012. A review of agricultural crop residue supply in Canada for Cellulosic ethanol production. *Renewable and Sustainable Energy Reviews* 16: 2954-2965.

13. Liu, T., B. McConkey, T. Huffman, S. Smith, B. MacGregor, D. Yemshanov and S. Kulshreshtha. 2014. Potential and impacts of renewable energy production from agricultural biomass in Canada. *Applied Energy* 130: 222 – 229.

14. Mani, S., L.G. Tabil and S. Sokhansanj. 2006. Effects of compressive force, particle size and moisture content on mechanical properties of biomass pellets from grasses. *Biomass and Bioenergy* 30: 648 – 654.

15. Mosier, N. S., C. Wyman, B. Dale, R. Elander, Y. Y. Lee, M. Holtzapple and M. R. Ladisch. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology* 96 (6): 673-686.

16. Naik, S.N., V. Goud, P.K Rout and A.K. Dalai. 2010. Production of first and second generation biofuels: a comprehensive review. *Renewable and Sustainable Energy Reviews* 14(2): 578-597.

17. Ohgren, K., R. Bura, G. Lesnicki, J. Saddler and G. Zacchi. 2007. A comparison between simultaneous saccharification and fermentation using steam-pretreated corn stover. *Process Biochemistry* 42: 834 – 839.

18. Perruzza, A.L. 2010. Exploring pretreatment methods and enzymatic hydrolysis of oat hulls. M.Sc. thesis. Toronto, Department of Chemical and Applied Chemistry, University of Toronto.

19. Quintero, J.A., L.E. Rincon and C.A. Cardona. 2011. Production of bioethanol from agro industrial residues as feedstocks. In *Biofuels: Alternative Feedstocks and Conversion Processes*, ed. A. Pandey, E. Gnansounou, C.G. Dussap, C. Larroche and S.C. Ricke, 253-260. Boulevard Langford Lane Kidlington, Oxford UK: Academic Press.

20. Rosillo-Calle, F. and L.A.B. Cortez. 1998. Towards proalcool II- a review of the Brazilian bioethanol program. *Biomass and Bioenergy* 14(2):115-124.

21. Sanchez, O.J and C.A. Cardona. 2008. Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresource Technology* 99(13): 5270 – 5295.

22. Sarkar, N., S. K. Ghosh, S. Bannerjee and K. Aikat. 2012. Bioethanol production from agricultural waste: An overview. *Renewable Energy* 37: 19 – 27.

23. Saskatchewan Agriculture, Natural Resources, and Industry. 2017. Oat Production and Market Factsheet. http://publications.gov.sk.ca/documents/20/84129-oat_production_and_markets.pdf (2017/06/02).

24. Saskatchewan Ministry of Agriculture field crop sheet. 2014. Field crop statistics. Accessed 11 December 2016.

25. Smith, D. 2013. Brief overview of biofuels. Report 2 and 3. McGill University, Montreal, QC: Biofuelnet Canada.

26. Sokhansanj, S., S. Mani, X. Bi, P. Zaini and L.G. Tabil. 2005. Binderless pelletization of biomass. In ASAE annual international meeting, paper no. 056061, St. Joseph, MI 49085 – 9659, USA.

27. Sorda, G., M. Banse and C. Kemfert. 2012. An overview of biofuel policies across the world. *Energy Policy* 2(38): 6977 – 88.

28. Tong, Z., P. Pullammanappallil and A.A. Teixeira. 2012. How ethanol is made from cellulosic biomass. AE493. Gainesville, Florida: Institute of Food and Agricultural Sciences, University of Florida.

29. Tumuluru, J.S., L.G. Tabil, Y. Song, K.L. Iroba and V. Meda. 2014. Grinding energy and physical properties of chopped and hammer-milled barley, wheat, oat and canola straws. *Biomass and Bioenergy* 60: 58 – 67.

30. United States Department of Agriculture, Foreign Agricultural Service. 2017. World Agricultural Production. https://apps.fas.usda.gov/psdonline/circulars/production.pdf (2017/06/02).

31. Van Zessen, E., M. Weismann, R.R.C. Bakker, H.W. Elbersen, J.H. Reith and H. den. Uil. 2003. Lignocellulosic ethanol, a second opinion. Report 2GAVE-03.11. http//library.wur.nl/WebQuery/wurpubs/ (2015/05/28).

Chapter 2

---

2. Microwave-Assisted Alkali Pretreatment and Enzymatic Saccharification Processes of Agricultural Crop Residues: A Review.

A similar version of this chapter has been submitted for presentation at the CSBE/SCGAB Annual General Meeting and Technical Conference joint with CIGR VI Technical Symposium International Conference of Canadian Society of Biological Engineers:

- Obiora S. Agu, Lope G. Tabil, Tim Dumonceaux and V. Meda. 2017. Microwave-assisted alkali pretreatment and enzymatic saccharification process of agricultural crop residues. CSBE/SCGAB 2017 Annual Conference, Paper No. CSBE17-122, Canad Inn Polo Park, Winnipeg, Manitoba, 6 – 10 August.

Contributions of M.Sc. Candidate

This literature review was collected, reviewed and developed to serve as a reference resource for information on the potentials of microwave-assisted alkali pretreatment techniques on lignocellulosic biomass, advantages of the microwave pretreatment technology and enzymatic saccharification processes used in the recovery of reducing sugars for bioethanol production. The whole manuscript was written by Obiora Samuel Agu, while Dr. Lope Tabil and Dr. Venkatesh Meda provided editorial input.

2.1 Abstract

The effect of microwave-assisted alkaline pretreatments and enzymatic saccharification of lignocellulosic agricultural crop residues are reviewed in this paper. Pretreatment is a major step for the efficient and effective conversion of lignocellulosic biomass to biofuel. Microwave-assisted alkali pretreatment is one of the promising techniques used in the bioconversion of biomass into useful energy product. The advantages of microwave heating coupled with alkaline pretreatment include: reduction of the process energy requirement, rapid and super heating, and low toxic compound formation. This paper reviews recent microwave-assisted alkali pretreatment and enzymatic saccharification techniques on different agricultural residues highlighting lignocellulosic biomass treatments and reducing sugars yields, and recovery. In

addition, compiled up to date research studies, development efforts and research findings related to the microwave-assisted alkali, and enzymatic hydrolysis are provided.

2.2 Introduction

With the prevailing challenges associated with fossil fuel usage, CO_2 emissions and energy security concerns, there is a problem of global warming due to green house gases (GHG) emissions from the combustion of fossil fuels and other energy uses. Due to this problem on the environment, research scientists are exploring ways of combating these emissions through renewable energy production such as bioethanol and biofuel (Nomanbhay et al. 2013; Diaz et al. 2015) which will reduce the over dependency on fossil fuels usage which results in emissions of sulfur dioxide (SO_2) and nitrogen oxides (NO_x), a major cause of acid rain. Fossil fuels are high in sulfur and nitrogen which form sulfur dioxide and nitrous oxides. These emissions result in acid rain which can damage fresh water sources, forests, soils and adversely affect human health (Demirbas 2004). Therefore, to overcome GHG emissions problems, there is a need to replace the existing energy source with a more reliable, renewable and environmentally friendly energy source. Lignocellulosic biomass feedstock is one potential source of renewable energy and also considered as a non-food material (second generation feedstock) as described in Figure 2.1 (Smith 2013). Bioethanol has a potential market value that is higher than gasoline, and can serve as a guarantee for transport fuel, generation of power and heat via combustion (Sarkar et al. 2012), and biochemical supply in the future for developing and industrialized nations (Demirbas et al. 2009; Smith 2013).
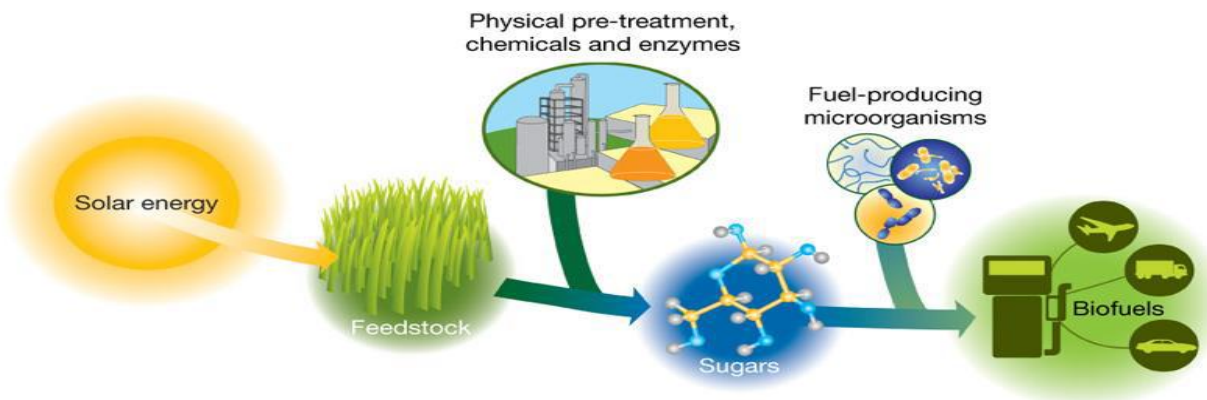
Figure 2.1. Ecosystem relationships to renewable energy production (Smith 2013).

2.2.1. Lignocellulosic Biomass

Lignocellulose biomass consists of cellulose, hemicellulose and lignin. Cellulose is the main molecule utilized for ethanol production. It is the main chemical component in lignocellulosic biomass. It is a linear polysaccharides chain of glucose units in the cell walls of plants (Bhaskar et al. 2011).

Cellulose comprises 40 – 50% of weight in plant biomass (Aho et al. 2013; Yin 2012) and has close to 10,000 degrees of polymerization in the biomass feedstock (Aho et al. 2013). They can form two glucose units fused together by a β-(1-4)- glycosidic linkage known as cellobiose and can form hydrogen bonds in between the polymer chains (Iroba and Tabil 2013). Also, the rigidity of the fibers in the cellulose which gives cellulose material strength is seen in the crystalline polymers of glucose (Yin 2012). Iroba and Tabil (2013) reported that the celluloses are in skeletal polysaccharides scattered in the cell wall components of agricultural residues/wastes, municipal cellulosic waste and wood feedstock.

Hemicellulose (15 – 30%) is enclosed in cellulose fibers and offers a structural linkage between cellulose and lignin. It is an amorphous and heterogeneous polysaccharide which is not chemically homogeneous unlike cellulose (Bhaskar et al. 2011).The polymer chains are shorter than those of cellulose and are described as water soluble because some sugar units are linked to the acetyl groups (Aho et al. 2013). Hemicellulose contains xylans. Hardwoods contain mostly hemicellulose xylans while softwoods contain more of glucomannans (Bhaskar et al. 2011).

Lignin is a very complex polymer that plays a cementing role in connecting plant cells and increases the mechanical strength properties of the plant against diseases and biodegradation (Karimi et al. 2013; Aho et al. 2013; Bhaskar et al. 2011). Also, with hemicellulose in an amorphous matrix, the cellulose fibrils are embedded and protected against biodegradation. Lignin acts as glue between hemicellulose and cellulose content while sometimes hemicellulose is referred as glue between lignin and cellulose (Karimi et al. 2013). Lignin is cross-linked with complex three- dimensional polymer of phenyl propane units connected by ether and carbon – carbon linkages (Iroba and Tabil 2013). Karimi et al. (2013) reported that during plant biosynthesis, lignin is not simply deposited between cellulose and hemicellulose but is linked with any part of them such as lignin-polysaccharide complex (LPC) or lignin-carbohydrate complex (LCC). These linkages make it almost impossible to separate lignin from cellulose and

hemicellulose, and to have lignin-free polysaccharides. Softwoods contain higher lignin (25 – 40 %) than hardwoods (18 – 25 %) and agricultural residue (10 – 20 %). The composition and content of lignin vary for different types of biomass materials (Aho et al. 2013; Bhaskar et al. 2011; Karimi et al. 2013). During a bioethanol processes, lignin is left as a residue. Although, it still has some energy value, which can be converted to variety of value-added products (Tong et al. 2012).

Table 2.1 Lignocellulose composition of agricultural biomass (Iroba and Tabil 2013; Sarkar et al. 2012; Harmsen et al. 2010).

Agricultural residues	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Baggase	65	(total carbohydrate)	18.4
Barely straw	40	20	15
Canola straw	42.39	16.41	14.15
Coastal bermudagrass	25	35.7	6.4
Corn cobs	45	35	15
Corn stalks	35	15	19
Corn straw	42.39	16.41	14.15
Cotton seed hairs	80 - 95	5 - 20	0
Grasses	25 - 40	35 - 50	10 - 30
Hardwood stems	40 - 55	24 - 40	18 - 25
Leaves	15 - 20	80 - 85	0
Oat straw	41	16	11
Nut shells	25 - 30	25 - 30	30 - 40
Rice hulls	36	15	20
Rice straw	32	24	13
Saw dust	55	14	21
Softwood stems	45 -50	25 - 35	25 - 35
Sorghum straw	33	18	15
Sorted refuse	60	20	20
Switchgrass	45	31.4	12
Wheat straw	30	50	15

Furthermore, lignocellulosic biomass contains other extraneous materials which are extracted by means of polar and non-polar solvents. These extraneous materials are grouped as

extractives and non-extractives. Their composition and content vary among the lignocellulosic biomass. Extractive components are resins (fats, fatty acids, resin acids and phytosterols), terpenes (isoprene alcohols and ketones), and phenols (residues and byproducts of lignin biosynthesis). Non-extractives are the inorganic components such as carbonates, oxalates, starches, pectins and proteins (Fan et al. 1982; Ramesh and Singh 1993; Karimi et al. 2013).

2.2.2 Bioethanol Processing

The production of bioethanol from agricultural residues looks more sustainable due to the utilization of non-edible portions of the crops. This minimizes competition with the food industry and increases the economic and social level of the community (Tong et al. 2012; Iroba et al. 2013). Sarkar et al. (2012) indicated that global bioethanol production in 2001 was 31 billion liters, in 2006 39 billion liters and was expected to reach 100 billion liters in 2015. These data showed that 62% of the production is from USA and Brazil. Also, the production of bioethanol from lignocellulosic biomass has been estimated to be 422 billion liters and 491 billion liters per year from crop residues and wasted crops. This is 16 times higher than the overall global bioethanol production (Sarkar et al. 2012). Global amount available for production of bioethanol from agricultural crop residues is represented in Table 2.2. Asia being the major producer of rice and wheat has the highest residues from the crops whereas corn straw and bagasse are produced more in America.

Table 2.2 Selected agricultural crop residues (megagram) reportedly available for bioethanol production (Sarkar et al. 2012).

Agricultural residue	Africa	America	Asia	Europe	Oceania
Rice straw	20.9	37.2	667.6	3.9	1.7
Wheat straw	5.34	62.64	145.2	132.59	8.57
Corn straw	-	140.86	33.9	28.61	0.24
Bagasse	11.73	87.62	74.88	0.01	6.49

Bioethanol can be blended with gasoline (E5, E10, and E85) taking advantage of the high octane number and higher heat of vaporization (Kim and Dale 2005) resulting from blended gasoline. Also, internal combustion engines operating on bioethanol generate fewer GHG since

bioethanol is less carbon-rich than gasoline (Nomanbhay et al. 2013). The efficient conversion of biomass feedstock to bioethanol fuel and scaling into industrial application is not yet fully achievable (Bram et al. 2005). This is because lignocellulosic biomass conversion technologies are still being researched and pilot-scale plants are faced with a lot of challenges. Werther et al. (2000) reported that the high cost of biomass conversion processes is limiting commercialization of the technology, thus making bioethanol production economically unattractive to the fuel market.

The lignocellulosic bioethanol process can be categorized into four steps: pretreatment, saccharification, fermentation, and product (ethanol) recovery (Quintero et al. 2011; van Zessen et al. 2003). Pretreatment helps in the breakdown of cell walls and internal tissues of the lignocellulosic biomass through biochemical conversion processes. This process involves disruption and disintegration of recalcitrant structures in order to open channels for enzymatic reactions processes in the material (Harsem et al. 2010; Mosier et al. 2005; Agbor et al. 2011) and Figure 2.2 shows the schematic effect of pretreatment. Each pretreatment technique employed has different effect on the various chemical components of lignocellulosic biomass (Alvira et al. 2010).
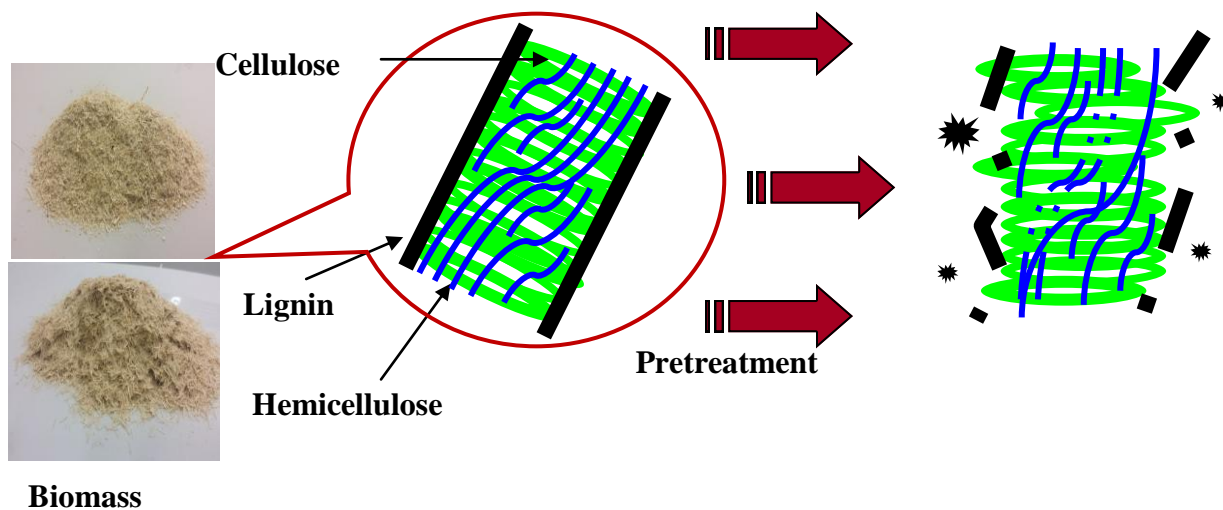
Fig 2.2. Effect of pretreatment on canola straw and oat hull.

Various pretreatment techniques have been developed such as alkali and microwave-assisted pretreatment, dilute acid, steam explosion, ammonia fiber explosion (AFEX), lime treatment and

organic solvent treatments, each technique assists in the breakdown of lignin to enhance saccharification of cell wall carbohydrates (Quintero et al. 2011).

2.2.2.1 Mechanical Pretreatment

Mechanical pretreatment process involves particle size reduction which increases the specific surface area and decreases degree of polymerization of the biomass (Kumar and Wyma 2010). Iroba and Tabil (2013), Fan et al. (2006), and Mohammad and Karimi (2008) reported that biomass particle size reduction facilitates inter-particle bonding. However, using the ball milling method, the small size reduction effectively transforms cellulose from crystalline to amorphous, decreases the degrees of polymerization of cellulose and hemicellulose, and increases the bulk density thereby improving the enzymatic saccharification. Karimi et al. (2013) reported that the increased degree of polymerization of cellulose is a major parameter contributing to low susceptibility of biomass to hydrolysis and in most cases, a decrease in the degree of polymerization of cellulose plays a more important role in hydrolysis compared to crystallinity. Also, Adapa et al. (2009) reported that the finer the grind size, the higher the quality of compact. The size reduction is done at different combinations of grinding and milling in order to improve enzymatic saccharification; however, the size reduction may not be feasible because of the high energy cost (Hendriks and Zeeman 2009; Alvira et al. 2010; Adapa et al. 2011), and it depends on the final particle size and biomass characteristics (Toma-Pejo et al. 2011).

2.2.2.2 Alkaline Pretreatment

The effect of alkali pretreatment on lignocellulosic biomass is dependent on the biomass lignin content (Alvira et al. 2010). Alkaline pretreatment improves cellulose digestibility, the ability to saponify intermolecular ester bonds, cross-linking xylan hemicelluloses and other components. It is more effective when combined with acid or hydrothermal processes (Carvalheiro et al. 2008). Furthermore, the advantage of alkali pretreatment is that some of the alkali reacts with the biomass components and the remaining residual alkali concentration will be involved in the reaction (Gossett et al. 1982). Sindhu et al. (2015) reported that when compared with acid, alkali pretreatment removes more lignin and reduces the degradation of carbohydrates in the biomass. The advantages of alkali pretreatment are: no washing of samples after pretreatment, no corrosion problem in the equipment used for the treatment as compared to acid,

and alkali pretreatment processes utilize lower temperatures and pressures compared with other pretreatment techniques (Sindhu et al. 2015; Karunanity and Muthukumarappan 2011). Also, alkali treatment processes show a high reaction rate on agricultural residues than on wood feedstock (Kumar et al. 2009).

Different alkalis suitable for the pretreatment are sodium, potassium, calcium and ammonium hydroxides (Sindhu et al. 2015). The sugar yield of alkaline pretreatment is dependent on the feedstock used. However, biomass used for the pretreatment process tends to react with some of the alkali, and it leads to solubilization, swelling, an increase in the internal surface of cellulose, a decrease in degrees of polymerization and crystallinity, and a disruption of lignin structure (Taherzadeh and Karimi 2008; Quintero et al. 2011).

However, a lot of research studies have reported that sodium hydroxide is commonly used as a pretreatment chemical for lignocellulosic biomass. This is due to its ability to delignify biomass. Thus, in large scale process, it may not be cost effective (Sindhu et al. 2015). Alkali pretreatment causes hemicellulose and some lignin parts to solubilize, and makes cellulose more accessible to enzymatic saccharification like in enzymatic hydrolysis of spruce (Zhao et al. 2008). When employed with NH_3, the alkali improved the digestibility of cellulose by decreasing crystallinity of the fibrils (Sindhu et al. 2015). Teymouri et al. (2005) reported that NH_3 depolymerizes the lignin at high temperature to breakdown polysaccharide matrix.

Also, KOH, when used with switch grass at very low concentrations was effective and generated high sugars during hydrolysis (Sharma et al. 2013). Kashaninejad et al (2010) reported that alkali pretreatment is a typical chemical pretreatment technique for lignocellulosic biomass based on the chemical reaction between alkali and lignocellulosic biomass.

2.2.2.3 Microwave Pretreatment Technology

Microwave (MW) irradiation is electromagnetic waves that consist of electric and magnetic fields. The waves are formed within a frequency band of 300 MHz and 300 GHz (Kappe et al. 2012). Motasemi and Afzal (2013) described three ways materials can be grouped according to MW irradiation: 1) MW-transparent material (insulator) where microwaves pass through without losses like teflon[TM] or quartz, 2) conducting material which cannot allow microwaves penetration but reflected like metals, and 3) absorbing materials like oil, water, etc.

MW has gained application in research studies because of its easy operation and high heating efficiency. MW heating is an alternative and energy efficient procedure when compared to conventional heating (Hu and Wen 2008). In comparison with conventional heating based on super critical heat transfer, MW has the ability to interact between heated object and an applied electromagnetic field directly, which subsequently increases the temperature of the object, thereby creating volumetric and rapid heating (Hu and Wen 2008; De la Hoz et al. 2005). MW pretreatment combines both thermal and non-thermal effects within the aqueous environment of physical, chemical or biological reactions (Ethaib et al. 2015).

The early discoveries of microwave pretreatment on lignocellulosic biomass was reported by Ooshima et al. (1984) and Azuma et al. (1984) (Hu and Wen 2008; Xu 2015) since then, the technology has shown an efficient applications in different ways such as reduction in process energy requirements, uniform and selective processing, gas and product (Gong et al. 2010; Keshwani and Cheng 2010; Quitain et al. 2013). The pretreatment of lignocellulosic biomass using MW heating is done selectively especially at the polar parts and this results in an increase in the disruption of the recalcitrant structures of the biomass (Tomas-Pejo 2011). Different pretreatments techniques to make lignocellulose accessible to enzymes for enhancing bioethanol conversion have been widely studied (Alvira et al. 2010). The MW pretreatment method shows effective results compared to other techniques as indicated in Table 2.3.

The combination of MW and chemical pretreatment on different feedstocks as reported by several research studies indicated higher sugar recovery, and various chemicals used in this process are: dilute ammonia, iron-chloride and the common ones, alkaline and acid. All these chemicals assist MW pretreatment technology in removing lignin (alkali solution) and hemicellulose (acid solution) for cellulose accessibility (Ethaib et al. 2015). The combined process separates lignocellulosic biomass components by disrupting the biomass structure, reducing the crystallinity of cellulose, improving the formation of fermentable sugars and reducing the degradation of carbohydrates (Sun and Cheng 2002). At lower temperatures, the combined pretreatment of lignocellulosic biomass improves enzymatic saccharification by accelerating the pretreatment reaction (Zhu et al. 2006; Hu and Wen 2008; Keshwani et al. 2007).

Table 2.3. Effect of different pretreatment technologies on the structure of lignocellulose (Tomas-Pejo et al. 2011).

Pretreatment	Increased Accessible Surface Area	Cellulose Decrystallization	Hemicellulose Solubilization	Lignin Removal	Lignin Structure Alteration	Toxic compounds Generation
Mechanical	H	H	0	0	0	0
Acid (Weak & Strong)	H	0	H	M	H	H
Alkai	H	H	M/H	H	H	L
Organosolv	M	0	H	M/H	M	M/L
Ozonolysis	M	M	M/H	H	M	L
Wet Oxidation	H	0	H	M	H	L
Microwave	H	H	L	H	H	L
LHW	H	0	H	L	M	L
AFEX	H	M	M/H	L	H	L
Steam Explosion	H	0	H	M	H	H
CO_2 Explosion	H	H	0/L	0	0	0
Ionic Liquids	M	H	H	M/H	M	M/L

H - High effect; M - Moderate effect; L - Low effect; 0 - No effect.

2.2.2.4 Enzymatic Saccharification

Enzymatic saccharification of lignocellulosic biomass usually takes place after the pretreatment process. This is a microbial degradation process, accomplished by using enzymes and the end result is usually sugar decrease (Quintero et al.2011). The saccharification process in the ethanol conversion is done in mild conditions at pH 5.2 – 6.2 and a temperature range of 45 – 50°C (Lan et al. 2012). There are three distinct major types of cellulase enzymes used in the process: 1) endoglucanases (E C 3.2.1.4) hydrolyses at random internal β-1, 4-glucosidic linkages in the cellulose chain producing oligosaccharides of different lengths and with shorter chain appearance; 2) exoglucanases of cellobiohydrolases (E C 3.2.1.91) progresses along cellulose chain ends and releasing major products as cellulose or glucose; and 3) β-glucosidases known as β-glucoside glucohydrolases (E C 3.2.1.21) hydrolyses cellulose to glucose, liberate cellobiose, soluble cellodextrins to glucose (Quintero et al.2011; Binod et al. 2011). Research studies reported different activities of enzymatic saccharification process with limiting factors on the lignocellulosic biomass such as moisture, available surface area, crystallinity of cellulose, degree of polymerization and lignin content (Alvira et al. 2010; Karimi et al. 2013; Chang and

Holtzapple 2000; Laureano-Perez et al 2005). In addition, Palonen et al. (2004) reported that the hemicellulose removal increases mean pore size of the biomass thereby increasing cellulose chances to get hydrolyzed. Consequently, lignin content reduces enzymatic saccharification by forming a shield and blocking substrate digestible parts from hydrolyzing (Chang and Holtzapple 2000).

This review is intended to identify the various microwave-assisted alkali pretreatment effects on the different lignocellulosic agricultural residues. Also, emphasis is placed on the pretreatment processes affects of the lignocellulosic biomass and its sugar yield/recovery from enzymatic saccharification.

2.3 Microwave-assisted Alkali Pretreatment Technique and Enzymatic Saccharification of Selected Lignocellulosic Agricultural Residues

The microwave pretreatment process has been described as most promising pretreatment technology that enhances and accelerates chemical reactions (Motasemi and Afzal 2013). As discussed in the previous section, the MW technology has shown an efficient applications in various ways such as reduction in process energy requirements, uniform and selective processing (Gong et al. 2010; Keshwani and Cheng 2010; Quitain et al. 2013), and has been successfully employed to pretreat different feedstocks (Xu 2015). In addition, MW technique in combined processes separate lignocellulosic biomass components by disrupting the biomass structure, reducing the crystallinity of cellulose, improve formation of fermentable sugars and reduce the degradation of carbohydrates (Sun and Cheng 2002). The combined pretreatments of lignocellulosic biomass improve enzymatic hydrolysis by accelerating the pretreatment reaction (Zhu et al. 2006; Hu and Wen 2008; Keshwani et al. 2007). Therefore, this section reviewed extensively the microwave-assisted alkali pretreatment methods and enzymatic saccharification processes of the different lignocellulosic agricultural residues.

2.3.1 Effect of Microwave Power, Alkali Concentration and Residence Time on Enzyme Digestibility.

Table 2.4 describes the effect of microwave-assisted alkali technology in enhancing enzymatic saccharification. Lignocellulosic biomass conversion to bioethanol is very challenging considering the heterogeneous nature of the feedstock used in the production. Microwave-assisted pretreatment is a process which leads to high lignin removal, improve the biomass morphology to facilitate the reactivity of the enzyme thereby increasing sugar yields (Merino-Perez et al. 2015; Chaturvedi and Verma 2013). Higher alkali pretreatment on lignocellulosic biomass increases cellulose digestibility and more effective for lignin solubilization (Tomas-Pejo et al. 2011); relatively long residence time is needed to produce high sugar yields at a lower temperature for alkali pretreatment technique. Microwave irradiation an effective heat source employed in alkali pretreatment to produce the temperature needed in the delignification of biomass to enable enzyme reactivity (Xu 2015). Microwave-assisted alkali pretreatment technology is effective depending on the lignin content of the feedstock. In order to obtain the optimal microwave-assisted alkali pretreatment condition that can improve enzymatic digestibility using various agricultural residues, different microwave power levels, residence times and alkali solutions of various concentrations were considered in this study.

Furthermore, microwave pretreatment technique has gained research attention and its future is growing yet still under laboratories development. The cost of microwave pretreatment is the main determinant associated with the adoption of this technology. Emphasis on the capital and operating cost investment will improve the pretreatment technique and hydrolysis process thereby reducing the cost of final product (bioethanol). Feedstock properties and reaction conditions are the two factors influencing microwave pretreatment characterization and yield of the final product. Sufficient data generated from the previous and recent studies can be used to quantify the dielectric properties of input biomass, to design and develop a continuous microwave-assisted pretreatment and enzymatic saccharification process unit for commercial scale-up. Future research studies on microwave-assisted pretreatment needs to consider combining biological treatment to enhance biomass digestibility. The concept is to develop a more economic pretreatment and hydrolysis techniques that can be sustainable and accepted by bioenergy industry.

Table 2.4. Summary effect of microwave power, residence time and alkali concentration in improving enzymatic digestibility.

	Biomass and Pretreatment (Alkali and Microwave)	Enzymatic Hydrolysis	Sugar Production (Yield and Quality	References
1.	Oil palm empty fruit bunch (EFB) fiber was washed and dried in an oven at 105°C for 24 h maintaining a constant dry weight. Particle size: 1-2 mm Alkali solution: NaOH of 3% (w/v) MW oven output power was 180 W. Residence time varied at three minutes intervals (3 – 21 min). After pretreatment, the samples were dried at 105°C for 4 h and composition components analyzed.	Enzymes used were *Trichoderma reesei* (E C. 3.2.1.4) and supplemented with β-glucosidase or cellobiase from *Aspergillus niger* (E C. 3.2.1.21). 5 g pretreated samples soaked in citrate phosphate buffer and placed in MW at 100 W for 4 h. • After the MW treatment, the sample was placed in water bath at 50^{0}C for 5min and the control samples were placed in a shaking water bath of 120 rpm. • After the control treatment, the samples were incubated at 50°C for 48 h and supernatants estimated for reducing using DNS method.	MW-assisted alkali optimum pretreatment condition for microwave-assisted alkali; • 180 W for 12 min (optimum component loss of the lignin and hemicellulose 74% and 24.5% respectively). Enzymatic saccharification results; • MW-assisted alkali: 411 mg of reducing sugar per gram EFB at cellulose enzyme dosage of 20 FPU. • MW-assisted alkali pretreatment and enzymatic hydrolysis 178 mg of reducing sugar per gram EFB.	Nomanbhay et al. 2013
2.	Switchgrass was oven dried at 70°C and Coastal Bermudagrass sun dried, and each sample ground in 2 mm sieve. Alkali solution: dilute NaOH, Na$_2$CO$_3$ and Ca(OH)$_2$ (0.1%, 2% and 3%) each. 5 g (dry basis) of each sample (1:10 ratio) in various concentrations. MW power level of 250 W. Residence time of 5min interval (5 to 20 min).	Enzyme used was *Trichoderma reesei* (E C. 3.2.1.4) and supplemented with β-glucosidase from *Aspergillus niger* (E C. 3.2.1.21). Sample compositions were determined by using NREL protocol method and sugar analysis were measured using DNS method.	NaOH microwave-based pretreated samples, • significantly higher in sugar yields compared to other alkalis. Switchgrass at optimal pretreatment condition, • 2% NaOH for 10 min showed 82% glucose and 63% xylose • Coastal bermudagrass with 1% NaOH at the same time showed 87% glucose and 59% xylose.	Keshwani and Cheng 2010.
3.	Corn straw and Rice were ground in a blender: particle sieve between 1-3 mm. 10 g of sample mixed in 60ml of different solutions of water: • 95% (v/v) glycerol-water • 95% (v/v) glycerol-NaOH 1.4M. • MW oven (Electrolux NE21S 2450 MHz-1300W) • Residence time: 2 min	Blender and sieved sample was cultivated with fungus *M. heterothallica* (potato dextrose agar) and hydrolyzed more by using commercial cellulase Celluclast (Novozymes). Reducing sugars produced were measured using DNS (miller 1959).	Corn straw pretreated in microwave alkali glycerol and aqueous glycerol solution showed, • lignin removal of 29.5% and 22.6%, and reduced cellulose and hemicellulose contents. Microwave pretreated rice husk (solid) immersed in water, • reduced hemicellulose and	Diaz et al. 2015

	• Infrared thermometer at 180°C used to measure temperature.		cellulose contents with increased lignin content. The microwave aqueous glycerol pretreated sample • 12% lignin removed, and hemicellulose and cellulose 43.5% and 12.3%, respectively. The highest saccharification yield was attained for both samples in alkaline glycerol.	
4.	Wheat straw; the simultaneous saccharification and fermentation (SSF) of microwave-assisted and conventional alkali pretreated wheat straw was used. Sample was cut to 1-2 cm, washed and air dried. 20 g of cut sample was immersed in 160ml: • 1% NaOH aqueous solution • MW oven at 700 W for 25 min. The same ratio for conventional treatment kept in 500 ml beaker for 60 min. • Dried residues: cut to 10-20 mesh size • Used as substrate for SSF process.	Cellulase enzyme from *Trichoderma reesei* was used and prepared using recommendation of commission on biotechnology IUPAC (Ghose 1987) and supplemented yeast used was *S. cerevisiae* YC-097. Analysis of sugar yield: DNS method. Samples were taken at regular intervals for the sugar measurement.	SSF optimal conditions of 100 g l^{-1} substrate, 40°C, 15 mg (cellulase) g^{-1} (substrate), initial pH 5.3 and 72 h for microwave-assisted alkali pretreated wheat straw gave an ethanol yield of 69.3% with ethanol concentration of 34.3 g l^{-1}. Conventional alkali pretreated wheat straw at SSF optimal conditions of 100 g l^{-1} substrate, 40°C, 20 mg (cellulase) g^{-1} (substrate), initial pH 5.3 and 96 h gave ethanol yield and concentration as 64.8% and 31.1 g l^{-1}.	Zhu et al. 2006
5.	Switchgrass: • air dried and milled in 0.85 mm mesh sieve and retained in 0.425 mm mesh sieve. Pretreatment: MW treatment and conventional heating. Sample was soaked in NaOH /water solutions; • 0.05 – 0.3 g alkali/g biomass at room temperature for 2 h. MW power level of 1000 W • setting #1 to #4. • #4 was used to heat the sample for 70 – 190°C in 30 – 120 s. • #1 maintaining the temperature with	Celluclast 1.5-L and Novozyme 188 (Sigma, St. Louis, MO) used enzymes and prepared using Ghose (1987) procedure. NREL protocol method (LAP 001, 002, 003 and 004) was followed in analyzing the sugar compositions.	The switchgrass soaked in water and treated with microwave; • total sugar yield of 34.5 g/100 g biomass from combined treatment at 58.5% of the maximal potential sugar yield. The highest yield 90% maximum potential sugars ; • 0.1 g/g of the alkali loading. The optimal conditions for microwave pretreatment; • 190°C, 50 g/L solid content at 30 min treatment time, • hydrolysis was 58.7 g/100 g biomass (99% potential maximum	Hu and Wen 2008

	fluctuation less than $\pm 2^{o}$C. The dried residue samples preserved for enzymatic hydrolysis while the filtrate reducing sugars were analyzed using NREL LAP 002 method. Similar procedure for conventional treatment. • oil bath presoaked (190°C for 5 min).		sugars).	
6.	Sugarcane bagasse; • dried and milled to screen size less than 1mm. Alkali solution; • 1% NaOH and H_2SO_4 at 10% biomass loading in solution were used for MW-alkali, MW-acid and MW-alkali-acid. MW oven power level; • 100, 180, 300, 450, 600, and 850 W Residence time varied from 1 – 30 min.	Enzyme used for the saccharification was commercial cellulase. The sample was analyzed using DNS as reported by Aswathy et al. (2010) and Sukumaran et al. (2009).	Generally, the result showed high recovery of fermentable sugar from sugarcane bagasse. MW-alkali and enzymatic hydrolysis gave the highest sugar yield • 0.665g/g dry biomass at 600 W for 4min MW-acid; • 0.249 g/g dry biomass at MW power of 100 W for 30 min. Mw-alkali-acid combined treatment • 1% NaOH and H_2SO_4 increased in reducing sugar yield 0.83g/g dry biomass Alkali pretreatment at 450 W for 5min showed about 90% lignin removal from the biomass.	Binod et al. 2012
7.	Wheat straw; • cut to 1 – 2cm length, washed and air dried. • Chemical compositions and moisture content were determined. 20 g of the dried sample was suspended in 160 ml • 1% NaOH solution in a 500ml beaker. • MW power 300, 500 and 700 W, • Residence time 15min to 2 h. Conventional alkali pretreatment, • boiling in a 500 ml beaker • 15 min to 2 h	Cellulase enzyme from *Trichoderma reesei* was used and prepared using recommendation of commission on biotechnology IUPAC (Ghose 1987). After the concentrations had reached plateau, residues were collected, washed, dried, and weighed. The hydrolysate analyzed for reducing sugars using DNS method.	At 700 W MW-alkali pretreatment for 25min, • sample showed weight loss of 48.8% and composition as 79.6% cellulose, 5.7% lignin and 7.8% hemicellulose After 60min conventional alkali pretreatment, • sample indicated weight loss of 44.7% and composition; 73.5% cellulose, 7,2% lignin and 11.2% hemicellulose.	Zhu et al. 2006a

	• after the both pretreatments, the residues were collected, washed neutral pH and dried at 65°C, and cut to 10-20mesh size, • composition analysis and enzymatic hydrolysis.			
8.	Sweet sorghum bagasse (SSB), • washed, dried at 50°C hot air, • cut milled through a 40 mesh screen • particle size reduced between 1 and 2 mm. 1 g biomass sample weighed into 125 ml Erlenmeyer flask with distilled and deionized water of 10 or 20 ml at different concentration of lime 0, 0.10, 0.15 or 0.2 g and mixed. • mixture placed in shaken water bath at 50°C for 5min, • MW (GE 2450 MHz) for 1000 W, • residence time of 2, 4, or 6 min.	Pretreated sample composition analyzed using, • NREL protocol method. Cellulase (ACCELLERASE 1500) was the enzyme used for the hydrolysis. Sugars were analyzed by using, • DNS method, • the calculation was based on a calibration curve developed from the standard glucose as reported by Liang et al. (2009).	SSB MW treated without lime showed a higher sugar recovery, • 39.8 g/100 g SSB equivalent to 65.1% of maximal total sugars SSB microwave lime treated, • 32.2 g/100 g SSB equivalent to 52.6% of maximal potential sugars.	Choudhary et al 2012.
9.	Ear of the wheat straw, • removed and milled to a particle size ≤ 2 mm, • maintained 92.5% dry matter. Orthogonal experimental design (L$_9$(3^4)) was used to optimize pretreatment conditions, • 1000 g in 2 L beaker containing NaOH solutions of 80, 100 and 120 g/kg involving nine pretreatments. MW power levels 400, 700 and 1000 W. Residence times of 5, 10 and 15 min. After pretreatment, • Samples separated into residue and liquid. • Residue was dried, stored at room temperature in 65% humidity, • liquid was stored in the freezer at – 4°C and later analyzed.	Pre-hydrolysis of the sample was carried out followed by the simultaneous saccharification and fermentation (SSF) of the sample. Enzyme used for the hydrolysis was Cellubrix L. Analysis of ethanol produced was determined as weight loss caused by CO_2.	Optimal pretreatment conditions using orthogonal analysis was observed in ratio of the biomass to liquid, • 80 g/kg, 10 kg/m^3 NaOH, • MW power of 1000 W, • residence time of 15 min. The ethanol yield was 148.93 g/kg higher than untreated wheat straw which was 26.78 g/kg.	Xu et al. 2011

10	Switchgrass, • oven dried and ground to a particle size of 1 mm in diameter, • stored in plastic bag at room temperature. MW oven at variable power levels ranging from 125 to 1250 W. Residence times of 5, 10, 15 and 20 min. Biomass loading, • 10% solid loading in dilute sulfuric acid (H_2SO_4), • sodium hydroxide (NaOH) at concentrations of 1, 2 and 3% (w/v) Placed in MW radiation at 250 watts.	Compositional analysis was done using NREL protocol method. The enzymatic hydrolysis was done using *Trichoderma reesei* (E C. 3.2.1.4) enzyme and supplemented with β-glucosidase or cellobiase from *Aspergillus niger* (E C. 3.2.1.21). Sugar analysis was done using DNS method (Miller 1959).	Sample immersed in 3% (w/v) NaOH with lower MW power level 250 W at 10 • highest reducing sugar yield compared conventional 60 min NaOH pretreatment of switchgrass. MW-H_2SO_4 gave low yield of reducing sugar.	Keshwani et al. 2007
11.	Rice straw, • cut to 1 – 2 cm length, washed, air dried, • chemical composition determined. 20 g of dried sample was immersed in 160ml of 1% NaOH solution. MW power level 300, 500 and 700 W. Residence time 15 min to 2 h. Alkali pretreatment; • The same ratio for sample and alkali solution, boiled in a 500 ml beaker (15 min to 2 h). Pretreated residues, • washed with tap water until neutral pH, and dried at 65^0C for 2 days, • weighed and cut to 10-20 mesh size, • chemical composition determined.	Cellulase enzyme from *Trichoderma reesei* was used and prepared using recommendation of commission on biotechnology IUPAC (Ghose 1987). After the hydrolysis, • residues were washed, dried, and weighed. • Moisture content measured to check the weight loss and chemical contents analyzed using HNO_3-ethanol, 72% (w/w) H_2SO_4 and two-brominating methods. Reducing sugars concentration were measured using DNS, glucose oxidase and chloroglucinol methods.	MW-alkali pretreatment effect, • at 700 W after 30 min the rice straw had a weight loss of 44.6%, cellulose 69.2%, lignin 4.9% and hemicellulose 10.2% Alkali-alone pretreatment, • 70 min; weight loss 41.5%, cellulose 65.4%, lignin 6.0% and hemicellulose 14.3%. Enzymatic hydrolysis, • MW-alkali pretreated showed higher hydrolysis rate and glucose content compared to alkali-alone pretreated.	Zhu et al. 2005
12.	Rice straw, • air dried and milled 0.850 mm mesh sieve and retained by a 0.450 mm mesh sieve. • Stored in a sealed plastic bag at room temperature, • chemical composition analyzed. MW power levels: 320, 500 and 680 W	Cellulase enzyme used was *Trichoderma reesei* and prepared with carboxymethyl-cellulase. After the hydrolysis, samples were collected, centrifuged and analyzed for sugar NREL protocol method.	Maximal efficiencies at optimal conditions , • 680 W at 24min, • substrate concentration 75 g/l showed increased yield of cellulose 30.6%, hemicellulose 43.3%, and total saccharification yield of 30.3%.	Ma et al. 2009

	Irradiation time of 20, 25 and 30 min. After the pretreatment, • slurry and residue separated (dried at 35°C).			
13.	Sweet sorghum juice, • extracted by crushing the stalks in a roller press. • The bagasse stored in sealed bag (20°C) at 20%. • Sample was milled through 18, 9.5, 6, 4, 2 and 1 mm mesh sieves of size, • separated into fractions of 9.5 – 18, 4 – 6 and 1 – 2 mm. 15 g dry weight of the samples pretreated in, • 28 % v/v solution of ammonium hydroxide and • water mixture in a ratio of 1:0.5:8 MW oven for 1 h at heating temperatures, • 100, 115, 130, 145 and 160°C to treat the biomass. Composition analysis on untreated and MW assisted dilute ammonia treated analyzed using, • NREL LAP (#42618, 42619, 42620, 42621. 42622) methods to estimate the sugar yield.	Enzyme used for hydrolysis, • Spezyme CP containing cellulase and Novozyme 188 and β-glucosidase, • yeast extract *Saccharomyces cerevisiae* (D$_5$A). Separate hydrolysis and fermentation (SHF) used for the enzymatic hydrolysis. NREL protocol method used to estimate; • sugar yields, • ethanol, • glycerol, • organic acids, • hydroxyl-methyl-furfural (HMF) and • furfurals.	Simon's stain method • increased the porosity of the biomass samples compared to untreated samples. The microwave-assisted dilute ammonia pretreatment • efficient in lignin removal by 48% at a comparatively low ammonia concentration, • low temperature and short time when compared with other pretreatment technologies. Glucose and ethanol yields among all the pretreatment conditions showed • 4.2 g glucose/10 g dry biomass, • 2.1 g ethanol/10 g dry biomass in 1 – 2 mm sieve size sorghum bagasse pretreated at 130°C for 1 h.	Chen et al. 2012
14.	Rice straw, • cut to 1 – 2cm, washed and air dried. Pretreatment combinations used; • MW/alkali, • MW/alkali/acid (2% H$_2$SO$_4$) and • MW/alkali/acid 1% NaOH containing 0.3% H$_2$O$_2$ stored in dark room and room temperature for 12 h. MW oven 300 W. Residence time 30 min.	Cellulase enzyme used was *Trichoderma reesei* and prepared using recommendation of commission on biotechnology IUPAC (Ghose 1987). After hydrolysis residues were collected, washed, dried, weighed and analyzed using DNS method.	Samples pretreated with • MW/alkali/acid/ H$_2$O$_2$ indicated highest in weight loss and cellulose content. • MW/alkali/acid and MW/alkali/acid/ H$_2$O$_2$ pretreatment recorded a good recovery of xylose. • MW/alkali/acid/ H$_2$O$_2$ had the highest hydrolysis rate and glucose content.	Zhu et al. 2006b

15.	Rice straw sample • cut into piece of size 1 – 2 cm and washed with tap water, and air dried. MW power ranged from 70 – 700 W Residence time of 1 – 5 min. • Approximately 5 g rice straw sample immersed in a 30 ml of NaOH (0.1 to 2%) concentration and left overnight. • Treated sample composition components were analyzed using NREL LAP-004 method.	E-CLEAN, endo-1, 4-β-glucanase from *Aspergillus niger* was used and supplemented with EBLUC and β-glucosidase from *Aspergillus niger*. After the hydrolysis, samples collected at different time intervals and analyzed for reducing sugars by using DNS method.	Optimum condition, • MW-assisted alkali pretreated samples had maximum reducing sugar (1334.79 μg/ml) compared to untreated samples. X-ray diffraction analysis showed that the crystallinity index of the MW-assisted alkali pretreated rice straw was significantly higher by 54.55% compared to untreated samples 52.2%.	Singh et al. (2014)
16.	Pineapple sample • washed in 0.1% sodium hypochlorite for 5, • peel and core waste screw pressed at 2.5 bar and formed cake, and stored at -22°C. 40 g of ground pineapple sample mixed with • 40 ml of NaOH 0.5 N • at room temperature for 1 h (Zhu et al. 2006; Hu and Wen 2008; Binod et al. 2012). MW pretreatment sample was vacuum filtered and the residue used for MW treatment. • MW power at 170, 340 or 510 W, and used 2.125, 4.25 and 6.375 W/g, • residence times of 5, 10, 20, 40, 60, 120 and 180 s. After the pretreatment, • samples turned power, • reconstituted with the filtrate, weighed and • stored for hydrolysis.	Cellulase (1.13 U/mg solid) 0.4% (w/w) and 0.1% (w/w) of hemicellulase (1.5 U/mg solid) from *Aspergillus niger* (L) were used with the pretreated pineapple waste and incubated at 50°C for 24 h. Sugars from MW pretreated and untreated samples were measured by high-performance anion-exchange chromatography with a pulsed amperometric detector (HPAEC-PAD).	MW power • 6.375 W/g for 5 s, 35.7% highest increase in fermentable sugar and 33.5% was the total sugar yield. Short residence times (up to 60 s) of MW heating gave a high yield of enzymatic hydrolysis compared to untreated pineapple waste. Increasing the MW heating time resulted in sugar degradation.	Conesa et al. (2016).

2.4 Summary and Prospects

Microwave-assisted alkaline pretreatment is a promising method which enhances enzymatic saccharification when employed on various agricultural crop residues and wastes. Conventional alkali heating method is also another traditional techniques applied to enhance enzymatic saccharification. This review identifies that microwave-assisted alkali pretreatment method can enhance the acceleration of enzymatic hydrolysis process compared to the conventional method. Sodium hydroxide solution identified as the most effective alkali compared to other alkalis. It was observed that NaOH, residence time and substrate concentration were main the factors affecting the enzymatic saccharification efficiency. From the different microwave-assisted alkali pretreatment methods, lower MW power and shorter exposure time of feedstock reactor improved enzymatic saccharification sugar yields. Lime was not a good alkali reagent for microwave pretreatment and enzymatic saccharification, whereas sodium and ammonium hydroxides were excellent with microwave pretreatment and enzymatic saccharification in high yields of sugars depending on the biomass used.

Apart from providing results from previous research studies, this review identified some challenges related to microwave alkali pretreatment and enzymatic saccharification process. The challenges are 1) selecting the accurate temperature sensor in order to avoid power level measurement error, and 2) choosing a proper alkali reagent for a particular biomass pretreatment in order to maximize high yield of reducing sugars.

2.5 References

1. Adapa, P., L. Tabil, G. Schoenau. 2009. Compaction characteristics of barley, canola, oat and wheat straw. *Biosystems Engineering* 104: 335 – 344.

2. Adapa, P., L. Tabil and G. Schoenau. 2011. Grinding performance and physical properties of non-treated and steam exploded barley, canola, oat and wheat straw. *Biomass and Bioenergy* 35: 549 – 561.

3. Agbor, V.B., N. Cicek, R. Sparling, A. Berlin and B.D. Levin. 2011. Biomass pretreatment: Fundamentals toward application. *Biotechnology Advances* 29: 675-685.

4. Aho, A., T. Salmi and D.Y. Murzin. 2013. Catalytic pyrolysis of lignocellulosic biomass. In *The Role of Catalyst for the Sustainable Production of Biofuels and Bio-chemicals,* ed. K. S. Triantafyllidis, A.A. Lappas and M. Stocker, 137-145. Netherlands: Elsevier.

5. Alvira, P., E. Tomas-Pejo, M. Ballesteros and M.J. Negro. 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. *Bioresource Technology* 101: 4851 – 4861.

6. Aswathy U. S., R. K. Sukumaran, G. L. Devi, K. P. Rajasree, R. R. Singhania and A. Pandey. 2010. Bio-ethanol from water hyacinth biomass: an evaluation of enzymatic saccharification strategy. *Bioresource Technology* 101: 925 – 30.

7. Azuma, J.I., F. Tanaka and T. Koshijima. 1984. Enhancement of enzymatic susceptibility of lignocellulosic wastes by microwave irradiation. *Journal of Fermentation Technology* 62(4): 377 – 384.

8. Bhaskar, T., B. Bhavya, R. Singh, D.V. Naik, A. Kumar and H.B. Goyal. 2011. Thermochemical conversion of biomass to biofuels. In *Biofuels: Alternative Feedstocks and Conversion Processes*, ed. A. Pandey, E. Gnansounou, C.G. Dussap, C. Larroche and S.C. Ricke, 51-77. Oxford, UK: Academic Press.

9. Binod P., K. Satyanagalakshmi, R. Sindhu, K. U. Janu, R. K. Sukumaran and A. Pandey. 2012. Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse. *Renewable Energy* 37: 109 – 116.

10. Binod, P., K.U. Janu, R. Sindhu and A. Pandey. 2011. Hydrolysis of lignocellulosic biomass for bioethanol production. In *Biofuels: Alternative Feedstocks and Conversion*

Processes, ed. A. Pandey, E. Gnansounou, C.G. Dussap, C. Larroche and S.C. Ricke, 229 – 250. Oxford, UK: Academic Press.

11. Bram, S., J. De Ruyck and A. Novak-Zdravkovic. 2005. Status of external firing of biomass in gas turbines. Process Institute Mechanical Engineering. *Power Energy* 219(2): 137 – 145.

12. Carvalheiro, F., L.C. Duarte and F.M. Girio. 2008. Hemicellulose biorefineries: a review on biomass pretreatments. *Journal of Scientific and Industrial Research* 67: 849 – 864.

13. Chang, V. and M.T. Holtzapple. 2000. Fundamental factors affecting biomass enzymatic reactivity. *Applied Biochemistry and Biotechnology* 84 - 86(1): 5 – 37.

14. Chaturvedi, V and P. Verma. 2013. An overview of key pretreatment processes employed for bioconversion of lignocellulosic biomass into biofuels and value added products. *Biotechnology* 3:415–43.

15. Chen, C., D. Boldor, G. Aita and M. Walker. 2012. Ethanol production from sorghum by a microwave-assisted pretreatment. *Bioresource Technology* 110: 190 – 197.

16. Choudhary, R., A.L. Umagiliyage, Y. Liang, T. Siddaramu, J. Haddock and G. Markevicus. 2012. Microwave pretreatment for enzymatic saccharification of sweet sorghum bagasse. *Biomass and Bioenergy* 39: 218 – 226.

17. Conesa C., L. Segui, N. Laguarda-Miro and P. Fito. 2016. Microwave-assisted alkali pretreatment for enhancing pineapple waste saccharification. *Bioresources* 11(3): 6518 – 6531.

18. De la Hoz, A., A. Diaz-Ortiz and A. Moreno. 2005. Critical review: microwaves in organic synthesis. Thermal and non-thermal microwave effects. *Chemical Society Review* 34: 164 – 178.

19. Demirbas, A., M.F.M. Balat and H. Balat. 2009. Potential contribution of biomass to the sustainable energy development. *Energy Conversion and Management* 50: 1746-1760.

20. Demirbas, A. 2004. 'Bioenergy, global warming, and environmental impacts, energy sources, part a: recovery. *Energy Sources* 26(3): 225 – 236.

21. Diaz, A.B., M.M. Moretti, C. Bezerra-Bussoli, C.daC.C Nunes , A. Blandino, R. da Silva and E. Gomes. 2015. Evaluation of microwave-assisted pretreatment of lignocellulosic biomass immersed in alkaline glycerol for fermentable sugars. *Bioresource Technology* 185: 316 – 323.

22. Ethaib, S., R. Omar, S. M. M. Kamal and D. R. A. Biak. 2015. Microwave-assisted pretreatment of lignocellulosic biomass: A review. *Journal of Engineering Science and Technology* 2(1): 97 – 109.

23. Fan, L., Y. Lee and M. Gharpuray. 1982. The nature of lignocellulosic and their pretreatment for enzymatic hydrolysis. *Advances in Biochemical Engineering Biotechnology* 23: 158 – 183.

24. Fan, L.T., L. Yong-Hyun and M. M. Gharpuray. 2006. The nature of lignocellulosic and their pretreatments for enzymatic hydrolysis. *Advances in Biochemical Engineering Biotechnology* 23: 157 - 187.

25. Ghose, T. K., 1987. Measurement of cellulase activities. *Pure Applied. Chemistry* 59(2) 257- 268.

26. Gong, G., D. Liu and Y. Huang. 2010. Microwave-assisted organic acid pretreatment for enzymatic hydrolysis of rice straw. *Biosystems Engineering* 107: 67 – 73.

27. Gossett, J.M., D.C. Stuckey, W.F. Owen and P.L. McCarty. 1982. Heat treatment and anaerobic digestion of refuse. *Journal Environmental Engineering* 437 – 454. Div. ASCE 108 (EE3).

28. Harmsen, P.F.H., W.J.J. Huijgen and L.M.B. Lopez. 2010. Literature review of physical and chemical pretreatment processes of lignocellulosic biomass. Energy research center of the Netherland. 10 (13): 1-49.

29. Hendriks, A.T.W.M. and G. Zeeman. 2009. Pretreatment to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology* 100: 10 – 18.

30. Hongtao L., R. P. Karen, X. Youmin and G. Ju'e. 2010. Comprehensive evaluation of effects of straw-based electricity generation: A Chinese case; *Energy Policy* 38:6153 – 6160.

31. Hu, Z. and Z. Wen. 2008. Enhancing enzymatic digestibility of switchgrass by microwave assisted alkali pretreatment. *Biochemical Engineering Journal* 38: 369 – 378.

32. Ibrahim, H. A. 2012. Pretreatment of straw for bioethanol production. *Energy Procedia* 14:542 – 551.

33. Iroba, K.L and L.G. Tabil. 2013. Lignocellulosic biomass feedstock characteristics, pretreatment methods and pre-processing of biofuel and bioproduct applications. US and

Canada perspectives. In *Biomass Processing, Conversion and Biorefinery*, ed. B. Zhang and Y. Wan, 61-98. New York, NY: Nova Science Inc.

34. Iroba, K.L., L.G. Tabil, T. Dumonceaux and S. Sokhansanj. 2013. Enzymatic saccharification of radio frequency alkaline pretreated and non-treated lignocellulosic biomass barley straw. Paper No. CSBE 13-016. Winnipeg, Manitoba: CSBE.

35. Iroba, K.L., L.G. Tabil, T. Dumonceaux and O. Baik. 2013. Effect of alkaline pretreatment on chemical composition of lignocellulosic biomass using radio frequency heating. *Biosystem Engineering* 116: 385-398.

36. Kappe, C. O., A. Stadler, D. Dallinger, R. Mannhold, H. Kubinyi and G. Folkers. 2012. Microwaves in organic and medicinal chemistry. John Wiley and Sons.

37. Karimi, K., M. Shafiei and R. Kumar. 2013. Progress in physical and chemical pretreatment of lignocellulosic biomass. In *Biofuels Technologies,* ed. V.K. Gupta and M.G. Tuohy, 53 – 96: Berlin, Germany: Springer.

38. Karunanity C. and Muthukumarappan K. 2011. Optimization of alkali, big bluestem particle size and extruder parameters for maximum enzymatic sugar recovery using response surface. *Bioresources* 6: 762 – 90.

39. Kashaninejad, M., B. Kashaninejad and L. Tabil. 2010. Effect of microwave pretreatment on densification of wheat straw. ASABE Paper No. 1009051. St. Joseph, MI: ASABE.

40. Keshwani, D.R. and J.J. Cheng. 2010. Microwave-based alkali pretreatment of switchgrass and coastal bermudagrass for bioethanol production. *Biotechnology Progress* 26(3): 644 – 652.

41. Keshwani, D.R., J.J. Cheng, J.C. Burns, L. Li and V. Chiang. 2007. Microwave pretreatment of switchgrass to enhance enzymatic hydrolysis. ASABE Paper No. 077127. St. Joseph, MI: ASABE.

42. Kim, S. and B.E. Dale. 2005. Environmental aspects of ethanol derived from no-tilled corn grain: non renewable energy consumption and greenhouse gas emission. *Biomass and Bioenergy* 28: 475 – 489.

43. Kumar, P., D. M. Barrett, M. J. Delwiche and P. Stroeve. 2009. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial and Engineering Chemistry Research* 48: 3713 – 3729.

44. Kumar, R and C.E. Wyman. 2010. Key features of pretreated lignocellulosic biomass solids and their impact on hydrolysis. *Bioalcohol Production* 3. 73 – 121. Biochemical Conversion of Lignocellulosic Biomass. https://doi.org/10.1533/9781845699611.1.73

45. Laureano-Perez, L., F. Teymouri. H. Alizadeh and B.E. Dale. 2005. Understanding factors that limit enzymatic hydrolysis of biomass. *Applied Biochemical and Biotechnology* 121: 1081 – 1099.

46. Li, X., E. Mupondwa, S. Panigrahi, L.G. Tabil, S. Sokhansanj and M. Stumborg. 2012. A review of agricultural crop residue supply in Canada for cellulosic ethanol production. *Renewable and Sustainable Energy Reviews* 16: 2954-2965.

47. Liang, Y., J. Yessuf, S. Schmitt, K. Bender and J. Bozzola. 2009. Study of cellulases from a newly-isolated thermophilic and cellulolytic Brevibacillus sp. Strain JXL. *Journal Industrial Microbiology Biotechnology* 36: 961 – 70.

48. Liu S. 2004. Analysis and measurement in papermaking industry. Chemical Industry Press, Beijing. Viewed May 18, 2016.

49. Ma, H., W. Liu, X. Chen, Y. Wu and Z. Yu. 2009. Enhanced enzymatic saccharification of rice straw by microwave pretreatment. *Bioresource Technology* 100: 1279 – 1284.

50. Merino-Perez, O., R. Martinez-Palou, J. Labidi and R. Luque. 2015. Microwave-assisted pretreatment of lignocellulosic biomass to produce biofuels and value-added products. In *Production of Biofuels and Chemicals with Microwave, Biofuels and Biorefineries*, ed. Z. Fang, R.L. Smith Jr., X. Qi, 197 – 223. Dordrecht, Netherlands: Springer.

51. McCleary B. V., I. Shammer, M. Glennie-Holmes. 1988. Measurement of $(1 – 3)$, $(1 – 4)$-β-D-glucan. *Methods in Enzymology* 160: 545 – 551.

52. Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry* 31: 420 – 428.

53. Mohammad, J.T. and K. Karimi. 2008. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. *International Journal Molecular Science* 9: 1621 – 1651.

54. Mosier, N. S., C. Wyman, B. Dale, R. Elander, Y. Y. Lee, M. Holtzapple and M. R. Ladisch. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology* 96 (6): 673-686.

55. Motasemi, F. and Afzal, M. T. 2013. A review on the microwave-assisted pyrolysis technique. *Renewable and Sustainable Energy* 28: 317 – 330.

56. Nomanbhay, S.M., R. Hussain and K. Palanisamy. 2013. Microwave-assisted alkaline pretreatment and microwave assisted enzymatic saccharification of oil palm empty fruit bunch fiber foe enhance fermentable sugar yield. *Journal of Sustainable Bioenergy System* 3: 7 – 17.

57. Ooshima, H., K. Aso, Y. Harano and T. Yamamoto. 1984. Microwave treatment of cellulosic materials for their enzymatic hydrolysis. *Biotechnology Letters* 6(5): 289 – 294.

58. Palonen, H., A.B. Bjerre, M. Tenkanen and L. Viikari. 2004. Evaluation of wet oxidation pretreatment for enzymatic hydrolysis of softwood. *Applied Biochemistry and Biotechnology* 117(1): 1-17.

59. Quintero, J.A., L.E. Rincon and C.A. Cardona. 2011. Production of bioethanol from agro industrial residues as feedstocks. In *Biofuels: Alternative Feedstocks and Conversion Processes*, ed. A. Pandey, E. Gnansounou, C.G. Dussap, C. Larroche and S.C. Ricke, 253-260. Oxford, UK: Academic Press.

60. Quitain, A.T., M. Sasaki and M. Goto. 2013. Microwave-based pretreatment for efficient biomass-to-biofuel conversion. In *Pretreatment Techniques for Biofuels and Biorefineries*, ed. Z. Fang, 117 – 130. Berlin, Germany: Springer.

61. Ramesh, C. K., and A. Singh, 1993. Lignocellulose Biotechnology: Current and Future Prospects. *Critical Reviews in Biotechnology* 13(2): 151-172.

62. Sanchez, O.J and C.A. Cardona. 2008. Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresurce Technology* 99(13): 5270 – 5295.

63. Sarkar, N., S. K. Ghosh, S. Bannerjee and K. Aikat. 2012. Bioethanol production from agricultural waste: An overview. *Renewable Energy* 37: 19 – 27.

64. Sharma, R., V. Palled, R. R. Sharma-Shivappa and J. Osborne. 2013. Potential of potassium hydroxide pretreatment of switch grass for fermentable sugar production. *Applied Biochemistry and Biotechnology* 169: 761 – 72.

65. Saskatchewan Ministry of Agriculture field crop sheet. 2014. Field crop statistics. www.publications.gov.sk.ca/redirect.cfm?p=74877&i=83776 (2015/07/20).

66. Sindhu R, A. Pandey and P. Binod. 2015. Alkaline treatment. In *Pretreatment of Biomass: Process and Technologies,* ed. A. Pandey, S. Negi, P. Binod and C. Larroche, 51 – 60. Oxford, UK: Elsevier.

67. Singh, R., S. Tiwari, M. Srivastava and A. Shukla. 2014. Microwave assisted pretreatment of rice straw for enhancing enzymatic digestibility. *Journal of Energy*. http://dx.doi.org/10.1155/2014/483813.

68. Sluiter, A., B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton and D. Crocker. 2004. Determination of structural carbohydrates and lignin in biomass. NREL/TP-510-42618. Golden, CO: Office of Energy Efficiency and Renewable Energy, National Renewable Energy Laboratory.

69. Smith, D. 2013. Brief overview of biofuels. Reports 2 and 3.Biofuelnet Canada. 1 – 57.

70. Somogyi N. 1952. Notes on sugar determination. *Journal of Biological Chemistry* 195: 19 – 23.

71. Sukumaran R., R. R. Singhania, G. Mathew and A. Pandey. 2009. Cellulase production using biomass feed stock and its application in lignocellulose saccharification for bioethanol production. *Renewable Energy* 34: 421 – 4.

72. Sun, Y. and J. Cheng. 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology* 83: 1 – 11.

73. Taherzadeh, M.J. and K. Karimi. 2008. Pretreatment of lignocellulosic waste to improve ethanol and biogas production: a review. *International Journal of Molecular Sciences* 9: 1621 – 1651.

74. Teymouri F., L. Laureano-Pereze, H. Alizadeh and B. E. Dale. 2005. Optimization of ammonia fiber explosion (AFEX) treatment parameters for enzymatic hydrolysis of corn stover. *Bioresources Technology* 96:2014 – 8.

75. Tomas-Pejo, E., P. Alvria, M. Ballesteros and M.J. Negro. 2011. Pretreatment technologies for lignocellulose to bioethanol conversion. In *Biofuels: Alternative Feedstocks and Conversion Processes,* ed. A. Pandey et al., 149-177. Oxford, UK: Academic Press.

76. Tong, Z., P. Pullammanappallil and A.A. Teixeira. 2012. How ethanol is made from cellulosic biomass. AE493. Gainesville, FL: Institute of Food and Agricultural Sciences, University of Florida.

77. Van Zessen, E., M. Weismann, R.R.C. Bakker, H.W. Elbersen, J.H. Reith and H. den. Uil. 2003. Lignocellulosic ethanol, a second opinion. Report 2GAVE-03.11. Wageningen, Netherlands: AFSG Biobased Products, Wageningen University and Research. http://library.wur.nl/WebQuery/wurpubs/339809 (2015/05/28).

78. Werther, J., M. Saenger, E.U. Hartge, T. Ogada and Z. Siagi. 2000. Combustion of agricultural residues. *Progress Energy Combustion Sciences* 26(1): 1 – 27.

79. Xu, J. 2015. Microwave pretreatment. In *Pretreatment of Biomass: Processes and Technologies*, ed. A. Pandey, 157 – 172. Waltham, MA, USA: Elsevier.

80. Xu, J., H. Chen, Z. Kadar, A.B. Thomsen, J.E. Schmidt and H. Peng. 2011. Optimization of microwave pretreatment on wheat straw for ethanol production. *Biomass and Bioenergy* 35: 3859 – 3864.

81. Yin, C. 2012. Microwave-assisted pyrolysis of biomass for liquid biofuels production. *Journal of Bioresource Technology* 120: 273-284.

82. Zhao Y., Y. Wang, J. Y. Zhu, A. Ragauskas and Y. Deng. 2008. Enhanced enzymatic hydrolysis of spruce by alkaline pretreatment at low temperature. *Biotechnology and Bioengineering* 99: 1320 – 8.

83. Zhu, S., Y. Wu, Z. Yu, X. Zhang, C. Wang, F. Yu and S. Jin. 2006. Production of ethanol from microwave-assisted alkali pretreated wheat straw. *Process Biochemistry* 41: 869 – 873.

84. Zhu, S., Y. Wu, Z. Yu, Q. Chen, G. Wu et al. 2006a. Microwave-assisted alkali pretreatment of wheat straw and its enzymatic hydrolysis. *Biosystems Engineering* 93(3): 437 – 442.

85. Zhu, S., Y. Wu, Z. Yu, J. Liao and Y. Zhang. 2005. Pretreatment by microwave/alkali of rice straw and its enzymatic hydrolysis. *Process Biochemistry* 40: 3082 – 3086.

Chapter 3

3. Microwave-Assisted Alkali Pretreatment and Densification of Canola Straw and Oat Hull.

Some portion of this chapter has been published in a peer-reviewed journal Bioengineering and presented at an international conference 2016 Annual General Meeting and Technical Conference of the Canadian Society of Biological Engineers:

- Obiora S. Agu, Lope G. Tabil and Tim Dumonceaux. 2017. Microwave-assisted alkali pretreatment, densification and enzymatic saccharification of canola straw and oat hull. *Bioengineering* doi:10.3390/bioengineering4020025
- Obiora S. Agu, Lope G. Tabil, Tim Dumonceaux and Charley Sprenger. 2016. Microwave-assisted alkali pretreatment and densification of canola straw and oat hull. CSBE/SCGAB 2016 Annual Conference, Paper No. CSBE16-033, Halifax World Trade and Convention Centre, 3 – 6 July.

Contributions of M.Sc. Candidate

The study established the baseline approach in the microwave-assisted alkali pretreatment and densification of canola straw and oat hull. The potential application of microwave pretreatment technique as a strategy to improve pelletization was explored. The quality of microwave-assisted alkali pretreated, microwave alone pretreated and untreated canola straw and oat hull pellets were assessed. These pellet characteristics (pellet density, tensile strength and dimensional stability) can be used by power generating station and biofuel industry (pellets producers) to perform relative comparison of pellet quality produced from any kind of agricultural residues. In addition, the pellet characteristics of microwave-assisted alkali pretreated, microwave alone pretreated and untreated canola straw and oat hull were used to evaluate the effects of microwave pretreatment process. The experimental design, data analysis, and writing of the journal manuscript were performed by Obiora Samuel Agu. Dr. Lope Tabil provided technical direction in experimental design, experimental resources (space and materials), data analysis and editorial input to this chapter. Dr. Tim Dumonceaux provided technical input, experimental resources for chemical composition and saccharification analysis and editorial input to this chapter. In

addition, Dr. Lope Tabil established research collaboration with Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatchewan where the chemical composition analysis was performed.

3.1 Abstract

The effect of microwave-assisted alkali pretreatment on lignocellulosic biomass of canola straw and oat hull was investigated. The ground canola straw and oat hull were immersed in distilled water, sodium hydroxide and potassium hydroxide solutions at two concentrations (0.75 and 1.5% w/v) and exposed to microwave radiation at power level 713 W and three residence times (6, 12 and 18 min). Bulk and particle densities of ground biomass samples were determined. Alkaline-microwave pretreated and untreated samples were subjected to single pelleting test in an Instron universal machine, preset to a load of 4000 N. The chemical composition analysis showed that microwave-assisted alkali pretreatment was able to disrupt and breakdown the lignocellulosic structure of canola straw and oat hull. The measured parameters, pellet density, tensile strength and dimensional stability were evaluated and the results showed that the microwave-assisted alkali pretreated pellets had a significantly higher density and tensile strength compared to samples that were untreated or pretreated by microwave alone.

3.2 Introduction

The world relies on fossil fuels for its energy usage and the sources of these fossil fuels are from coal, oil and natural gas. Any event that threatens their availability affects the cost of supply such as is presently being experienced in petroleum supply (Nomanbhay et al. 2013). However, the negative impact of fossil fuels on the environment is the increasing problem of greenhouse gas emissions. These emissions to the environment have attracted global interest in searching for alternatives, non-petroleum based sources of energy (Nomanbhay et al. 2013; Alvira et al. 2010). These renewable energy sources include solar energy, biomass, wind, hydroelectric and other sources which are more environmental friendly (Balat et al. 2008).

According to Alvira et al. (2010) and Balat et al. (2008), fuel ethanol can be produced from renewable biomass such as sugar, starch or lignocellulosic materials. It is clear that lignocellulosic materials from agricultural residues are an interesting alternative. This is because the second and third generation feedstocks have been attracting research interest, less expensive

38

than conventional agricultural feedstocks, available worldwide, do not compete with food crops (non-food materials), are renewable and a good source of raw materials for developing bio-based products and bio-chemicals such as bioethanol or biodiesel (Demirbas et al, 2009; Smith 2013). Lignocellulosic materials include agricultural residues and by-products such as canola straw, wheat straw, rice straw, oats straw, corn stover, corn fiber, oat hull, rice hull, etc (Mosier et al. 2005). According to Sanchez and Cardona (2008), annual production of lignocellulosic biomass residue was estimated at 1×10^{10} megagram (Mg) word-wide. In Canada, the estimated average agricultural residue generated for over a 10 year period (2001 – 2010) was 82. 35 million dry Mg/y and Saskatchewan recorded the highest at 17.38 million dry Mg/y (Li et al. 2012). These agricultural residues and by-products can be used for conversion into bioethanol.

Canola and oat are major crops grown in Canada. Canola, an oilseed has an estimated crop production of 15,555.1 Mega gram per year (Mg/y) and Saskatchewan production is estimated at 8.9 Mg/y. While oat production is estimated to be 2,907.5 Mg/y and Saskatchewan (1.6 Mg/y), Manitoba and Alberta are the major producers in Canada (Sask. Min. of Agric. Field Crop Sheet 2014).

The pretreatment of lignocellulose material from agricultural residue is a key step for efficient utilization of biomass for ethanol production. Pretreatment helps in the breakdown of cell walls and internal tissues of the lignocellulosic biomass through biochemical conversion processes involving disruption and disintegration of recalcitrant structures in order to open channels for enzymatic reactions processes in the material (Mosier et al. 2005; Agbor et al. 2011; Quintero et al. 2011). An effective pretreatment technique is needed to liberate the cellulose from lignin, reduce cellulose crystallinity and increase cellulose porosity (Nomanbhay et al. 2013; Zhu et al. 2006; Zhao et al. 2008). Various pretreatment methods have been developed, but the choice of pretreatment technology for a particular raw material is influenced by many factors such as enzymatic hydrolysis step and enzymes used (Alvira et al. 2010). Such pretreatment methods include; alkali and microwave-assisted pretreatment dilute acid, steam explosion, ammonia fiber explosion (AFEX), lime treatment and organic solvent treatments. Also, combinations of these methods have been studied and are still ongoing (Alvira et al. 2010).

Microwave pretreatment method is a physico-chemical process involving thermal and non-thermal effects. Microwave has gained application in research studies because of its easy

operation, high heating efficiency, reduction of process energy requirements, selective heating, etc. The early discoveries of microwave pretreatment on lignocellulosic biomass were reported by Ooshima et al. (1984) and Azuma et al. (1984) and since then, the technology has shown efficient applications in various ways (Gong et al. 2010; Keshwani and Cheng 2010; Quitain et al. 2013). Microwave-assisted alkali pretreatment breaks down the lignocellulosic biomass components by disruption of biomass structure, reduction in crystallinity of cellulose, improvement in the formation of fermentable sugars and reduction of the degradation of carbohydrates (Sun and Cheng 2002). The pretreatment process is carried out by immersing the biomass in alkaline solution and exposing the slurry to microwave radiation for varying residence time (Keshwani and Cheng 2010). Research studies reported that alkaline reagents (sodium hydroxide) are the most effective and suitable for microwave-assisted pretreatment (Zhu et al. 2006; Alvira et al. 2010). Kashaninejad and Tabil (2011) investigated the effect of microwave pretreatment on the densification of wheat straw using dilute NaOH and $Ca(OH)_2$ solutions. The results indicated that the density and tensile strength of microwave alkali pretreated pellets were significantly higher than the untreated samples. Xu (2015) reported on microwave/water alone pretreatment on milled barley, spring wheat, winter wheat and oat straw for biogas. The results indicated that there was no improved yield on the anaerobic digestion of the biomass materials used and concluded that microwave pretreatment may not be appropriate for milled straw varieties in biogas plants. The first study on the use of microwave heating for pretreatment was carried out on rice straw and bagasse reported by Ooshima et al. (1984). The result showed increased enzymatic accessibilities by 1.6 and 3.2 times for rice straw and bagasse compared to untreated samples. In addition, Rodrigues et al. (2011) evaluated the potential of microwave-assisted alkali pretreatment to improve the rupture of the recalcitrant structures of cashew apple bagasse and the results indicated that microwave residence time and power had no significant effect on the glucose yield. Combining microwave pretreatment with ammonia successfully resulted in 48% delignification of sorghum bagasse at very low ammonia concentrations, reduced temperature and very short pretreatment time compared with other technologies (Xu 2015). Microwave-assisted lime and microwave-alone pretreatments were compared on wheat straw by Saha et al. (2008). Total sugar per gram straw released after enzymatic hydrolysis was achieved from microwave-assisted lime pretreatment at lower

concentration and temperature, and short pretreatment time higher than microwave-alone pretreatment.

Biomass feedstock is bulky, loose and difficult to utilize as a fuel. The biomass has high moisture content, irregular shape and size, and low bulk density. All these factors make it difficult to handle, transport, store and utilize the biomass feedstock in its original form (Adapa et al. 2013). Some agricultural straws can be turned into forage by ensiling or made into pellets for energy applications. Pelletizing of biomass is a primary means to achieve densification (Veal 2010). Densification increases the density of final pellet product to 600 – 1200 kg/m^{-3} (Kashaninejad and Tabil 2011; Mani et al. 2006) for efficient transport and storage, and low moisture content (8% w.b) for safe storage (Mani et al. 2006). Densification of biomass, such as pelletizing or briquetting increases bulk density, improves handling and storage characteristics, enhances volumetric calorific value, reduces transportation cost, improves combustion process control with coal, gasification and pyrolysis, increases uniformity of physical properties (shape and size) (Jenkins et al. 2011; Kashaninejad and Tabil 2011). Cellulose, lignin, hemicellulose, extractives and non-extractives are components of lignocellulosic biomass.

However, it was observed from the research studies that there is knowledge gap in the application of microwave-assisted alkali pretreatment and densification on canola straw and oat hull. Therefore, the objective of this research was to investigate the effect of microwave-assisted alkali pretreatment on the densification characteristics of canola straw and oat hull.

3.3 Materials and Methods

3.3.1 Sample Preparation

Two agricultural residues (canola straw and oat hull) were used in this study. The canola cultivar was a hybrid variety (LL252) and harvested on September 23, 2015 on NW 02-43-13-W3 100 km NW of Saskatoon in the Black Soil Zone. The canola straw was collected from the same zone and oat hull was sourced from (Richardson Milling Ltd.) Martensville, Saskatchewan (52.29^{o}N, 106°W).

3.3.2 Experimental Set-up

An overview of the experiment set-up is shown schematically in Figure 3.1. After the grinding, the samples physical properties as received were determined and the ground samples subjected to microwave-assisted alkali pretreatments. The sample slurries were dried and conditioned to 12% moisture content required for densification. The physical and chemical properties of the pretreated samples were determined and the data evaluated from canola straw and oat hull pellets were analyzed.

```
                        ┌─────────────────────────┐
                        │ Canola straw and oat hull│
                        └─────────────────────────┘
                                    │
                        ┌─────────────────────────────────┐
                        │ Hammer mill and aspirator cleaning│
                        │   (1.6 and 3.2 mm screen size)    │
                        └─────────────────────────────────┘
          ┌─────────────────────┐         │
          │ Physical properties │         │
          │ (As-received samples)│        │
          └─────────────────────┘ ┌──────────────────────┐
                                  │ MW/alkali pretreatment │
                                  └──────────────────────┘
                                           │
                                  ┌──────────────────────┐
                                  │ Slurry conditioning   │
                                  └──────────────────────┘
          ┌─────────────────────┐          │
          │ Physical properties │  ┌──────────────────┐
          │ 1.  Ash content     │  │  Densification    │
          │ 2.  Bulk density    │  └──────────────────┘
          │ 3.  Particle density│          │
          │ 4.  Moisture content│  ┌──────────────────────┐
          └─────────────────────┘  │ Pellets quality analysis│
                    │              │ 1.  Pellet density     │
          ┌─────────────────────┐  │ 2.  Dimensional stability│
          │ Chemical properties │  │ 3.  Tensile strength   │
          └─────────────────────┘  └──────────────────────┘
```

Figure 3.1. Flow diagram of the experimental procedure.

3.3.3 Cleaning, Grinding and Moisture Analysis

The canola straw was ground using a hammer mill (Glen Mills Inc. Clifton, NJ, USA) powered by a 1.5 kW electric motor with a screen opening size of 1.6 and 3.2 mm. The oat hull was cleaned using an aspirator cleaning machine (Carter-Day Company N.E Minneapolis, MN, USA) to remove some oat kernel remaining after initial cleaning by the producers. The cleaned

oat hull was ground using the same hammer mill and screen opening sizes. Figure 3.2 shows the as received and ground samples. A dust collector including a cyclone system was used to collect the ground samples and reduced the dust during operation. The moisture contents of samples as-received and ground were determined using ASABE standard S358.2 (2006) in three replicates and moistures are expressed in percent wet basis (% w.b.).
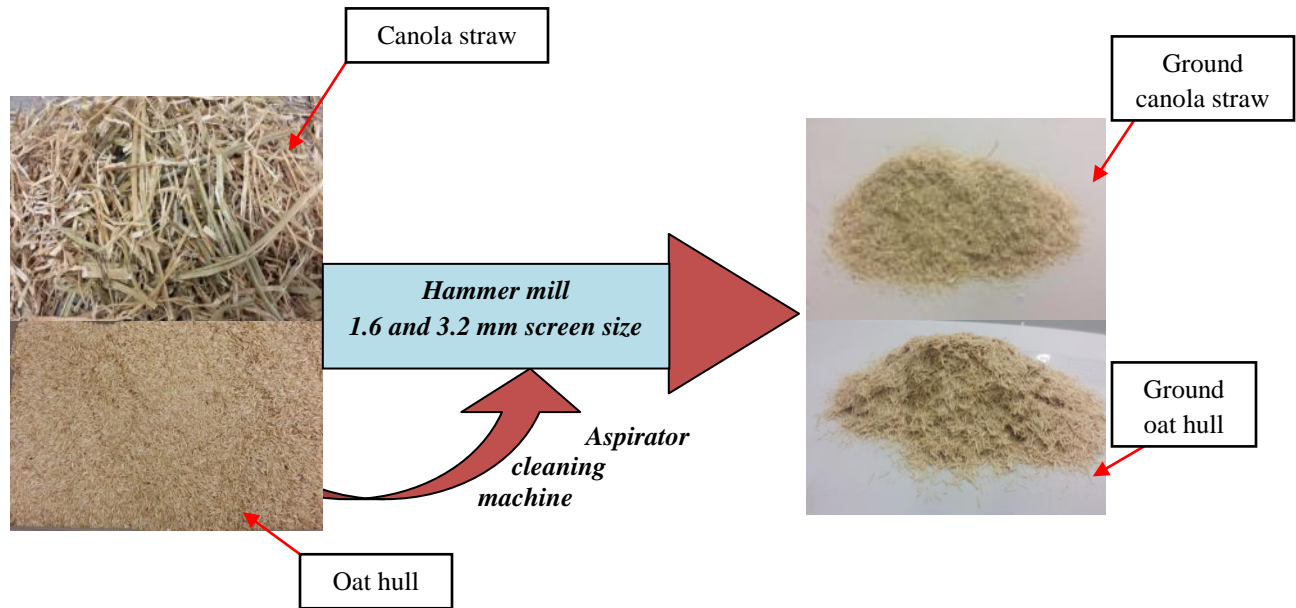
Figure 3.2. Ground canola straw and oat hull.

3.3.4 Bulk and Particle Density Analysis

The bulk densities of pretreated and untreated ground samples were determined and calculated using the mass and volume of a standard cylindrical steel container with 0.5 L volume (SWA951, Superior Scale Co. Ltd., Winnipeg, MB, Canada). The sample passed through a funnel and filled the 0.5 L volume container. A thin steel rod was used to roll across the sample on top of the container in a steady pattern motion and after, the sample together with the container was weighed. The particle densities of the treated and untreated ground samples were determined. Ground canola straw and oat hull of known mass were placed in the gas multi-pycnometer (QuantaChrome, Boynton Beach, FL, USA) and the volume of the sample determined. Thereafter, the particle densities were calculated by mass per unit volume of the

samples following the method reported by Adapa et al. (2009). The procedure was done in five replicates for both bulk and particle density measurements.

3.3.5 Particle Size Analysis

The particle size analysis of the ground samples was determined before microwave-assisted alkali pretreatment and densification. The geometric mean particle diameter of ground sample canola straw and oat hull was determined using ASAE Standard S319 (2008). The geometric mean diameter (d_{gw}) of the sample and geometric standard deviation of particle diameter (S_{gw}) were calculated using the standard mentioned (Mani et al. 2006, Adapa et al 2009 and 2011).

3.3.6 Microwave Pretreatment

Microwave (MW) treatments were carried out using a domestic microwave oven (Model NNC980W, Panasonic Canada Ltd, Mississauga, ON, Canada) with an operating frequency of 2450 MHZ and variable power from 220 to 1100 W. The microwave heating temperature data recording and acquisition in the experiment was done using Qualitrol Corporation software and the Nomad Fiber Optic Thermometer (Model NMD228A, Quebec City, QC, Canada). The data logging was one data point for every 5 s. Twenty grams of ground biomass sample (canola straw and oat hull) was immersed in 180 g of various alkaline solutions of 0, 0.75 and 1.5% (w/v) NaOH and 0, 0.75 and 1.5% (w/v) KOH. The mixture was placed in a 600 ml beaker and biomass mixture allowed to absorb the alkaline solution for a period of 30 to 45 min. The mixture was placed at the center of rotating ceramic plate inside the microwave oven for treatment at a fixed power of 713 W (Kashaninejad and Tabil 2011).

The temperature probe was inserted through a hole closed with a cork on top of the microwave oven and inserted half way into the beaker containing the sample. The mixture was exposed to three levels of residence time 6, 12 and 18 min, and temperature reading recorded accordingly. The process was done in five replicates for each sample. Figure 3.3 shows the experimental set-up. At every interval of 3 min heating, the microwave was stopped, and the beaker taken out and stirred for few seconds. This is to ensure uniform heating within the reactor. After the treatments, the moisture content of each sample was determined. The samples were

dried and maintained at appropriate moisture level of 12% (w.b.) using forced-air convection dryer set at 42^oC (Iroba and Tabil 2013) and stored in Ziploc bag.
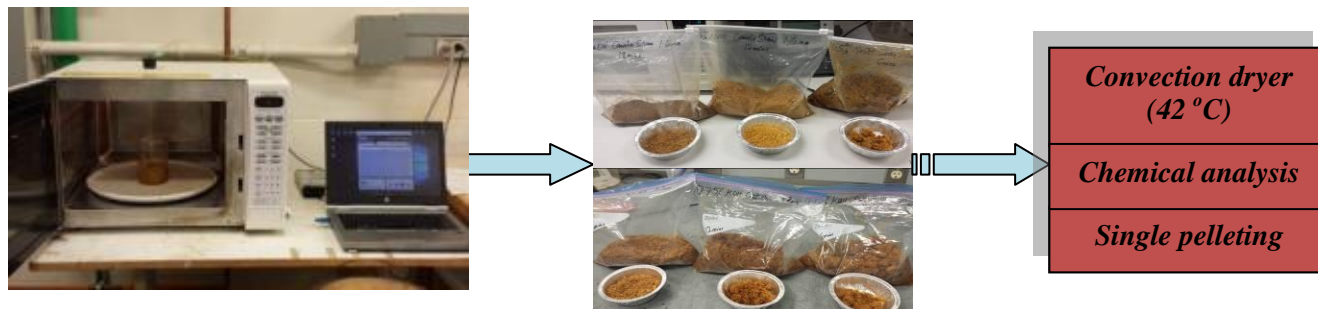
Figure 3.3. Glass beaker reactor containing the biomass-alkali solution mixture with the temperature probe fiber in the microwave pretreatment set-up and the pretreated samples.

3.3.7 Chemical Analysis

The chemical composition analysis of microwave-alkali pretreated and microwave pretreated canola straw and oat hull was performed using the National Renewable Energy Laboratory (NREL) standard (Sluiter et al. 2007). The samples selected for the analysis were based on pellet characteristics (tensile strength, dimensional stability and pellet density) that describe pellet quality. The analysis protocol according to NREL was done in a two-stage acid hydrolysis, with 72% H_2SO_4 and 4% H_2SO_4, in order to fractionate the biomass into forms that are quantifiable (Iroba and Tabil 2013). Prior to this analysis, the biomass samples at 11 – 12% (wb) were dried at 105^oC in an air-oven (Thermo Science model No. PR305225M; Marietta, OR, USA.) for 24 h. The extractive removal was done by adding the sample to a filter paper pouch, refluxed with acetone using a Soxhlet apparatus for 24 h. The acetone washed sample was left at room temperature for about 3 – 4 h in order to allow acetone to evaporate and then followed by oven drying at 105^oC for 24 h. And 300 mg of each oven dried extractive free sample was added into a 100 ml glass pressure tube and 3 ml of 72% H_2SO_4 was added. The mixture was macerated with glass rod every 10 min for 2 h. The acid mixture was diluted to 4% by adding 84 ml of distilled water and autoclaved for 2 h. The sample was allowed to cool at room temperature and separated using side-arm flask vacuum filtration device into hydrolysate (soluble material) and retentate (insoluble material). The retentate inside the crucible of each sample was dried at 105^oC

for 24 h and subsequently cooled in a desiccator before the weight was determined and recorded. Acid insoluble lignin content was evaluated based on the NREL protocol as presented in equation (3.1):

$$\text{Insoluble lignin content} = \frac{(dried\ retentate)}{(dried\ sample)} \text{ x } 100\% \qquad (3.1)$$

The acid soluble lignin was measured using UV – Vis spectroscopy (BIOMATE 3S, Thermo Fisher Scientific, Madison, WI, USA) at an absorbance of 240 nm. 30 ml of the hydrolysate was neutralized by adding 1 g of $CaCO_3$ and mixed. The mixture was allowed to settle for 5 min and 10 ml of the liquid fraction was carefully poured into a 15 ml centrifuge and subsequently centrifuged at 150 rev/min for 2 min, 1 ml of cleared supernatant was collected and stored at -20°C before monosaccharide quantifications using the Water Acquity UPLC – MS system (Acquity 2004 – 2010, Water Corp., Milford, MA, USA). Sample preparation for monosaccharide quantification was: 100 µl of stored neutralized hydrolysate with 800 µl of 75% acetonitrile/25% methanol and 100 µl of fucose solution (~1 mg/ml) and filtered through 0.2 µm filter into a 2 µl UPLC vial. The LC conditions for the monosaccharide quantification were: Acquity UPLC BEH Amide column (1.7 µm pore size, 2.1 X 50 mm); 0.25 ml min^{-1} flowrate; mobile phase A: 95% acetonitrile/5% isopropanol; mobile B: 80% acetonitrile/0.1% NH_4OH; gradient of 100% A to 100% B over 10 min, then gradient of 100% B to 100% A over 4 min (14 min total run time per sample). The UPLC - MS conditions for the same monosaccharide quantification were: 2.8 KV; 25 V (cone); 50 1 h^{-1} (cone); gas flow 600 1 h^{-1}; desolvation temperature 350 ^{o}C; source temperature 120°C; and dwell time 0.08 s.

At the concentrated acid stage, the polymeric carbohydrates (cellulose and hemicelluloses) were hydrolyzed into monomeric forms (xylose, arabinose, mannose, glucose and galactose), soluble in the hydrolysis liquid and were measured by UPLC. The standards of the monomeric sugars were prepared and evaluated using the UPLC. The spectra of mannose, glucose, and galactose displayed at a molecular weight 179.2 g/mol while xylose and arabinose displayed at a 149.1 g/mol. The correlated monosaccharide peak extracted from the integrated peak area was used to pre-determine regression equations from dilution series of the monosaccharide standards using Microsoft Excel.

The monomeric sugars regression analysis was determined using regression approach, the sugar content evaluated as:

$$\text{Sugar content} = \frac{sugar\ concentration \times 87\ ml \times 10 \times H}{dried\ sample\ mass} \text{ x } 100\% \qquad (3.2)$$

where, H could be 0.88 or 0.90 depending on the number of carbons present in the sugars which accounts for the water molecule added during the hydrolysis. 5-carbon sugars (pentoses: xylose and arabinose) and 6-carbon sugars (hexoses: mannose, galactose and glucose) values were multiplied by anhydro correction factor of 0.88 and 0.90, and replicated three times for each sample.

3.3.8 Ash Content

Ash content is a measure of mineral content and extractable in biomass (Iroba and Tabil 2013). The ash contents of canola straw and oat hull were determined based on National Renewable Energy Laboratory standard (Sluiter et al. 2008). About 2.0 ± 0.2 g of the oven dried microwave alkali treated and untreated samples were weighed into the tared dried crucible. The weighed crucible and sample were placed in a muffle furnace (Model F-A1T30, Thermolyne Sybron Corp., Dubuque, IA, USA) and baked overnight at $575 - 600^{o}$C. The sample was removed placed in an oven 105°C for $20 - 30$ min before being placed in a desiccator to cool. Ash content was determined gravimetrically. The ash content was calculated as the percentage of residue remaining after drying and each sample was replicated three times.

3.3.9 Densification

The microwave-assisted alkali pretreated and untreated samples were compressed and pelleted in a single pelleting unit consisting of a plunger-cylindrical die connected to a computer that interprets and records the force-displacement data (Figure 3.4). The plunger was connected to the Instron universal machine (Model 3366R4848 Instron Corp. Norwood, MA, USA) in which the upper moving crosshead provided the load necessary to compress the biomass samples. About $0.5 - 0.8$ g of selected pretreated and untreated biomass samples was loaded into the die cylinder. The temperature adjusted at about 95°C and a pre-set load compressed the samples. A 5000 N load cell fitted Instron universal machine was used and a pre-set load of 4000 N compressed the samples. The plunger compressed the biomass sample using a crosshead speed of 50 mm/min. Once the pre set load was achieved, the plunger was stopped and held in position for 60 s to avoid spring back effect of biomass (Mani et al. 2006; Kashaninejad and Tabil 2011).

Ten pellets were produced each from pretreated and untreated biomass samples. The force-deformation data at compression and force-time data at stress relaxation were recorded. The physical characteristics of the densified pellets such as pellet density, dimensional stability, and tensile strength were determined to evaluate the effect of the treatment combinations.

Internal pelleting die Chamber

Single pellet test setup

Canola straw and oat hull
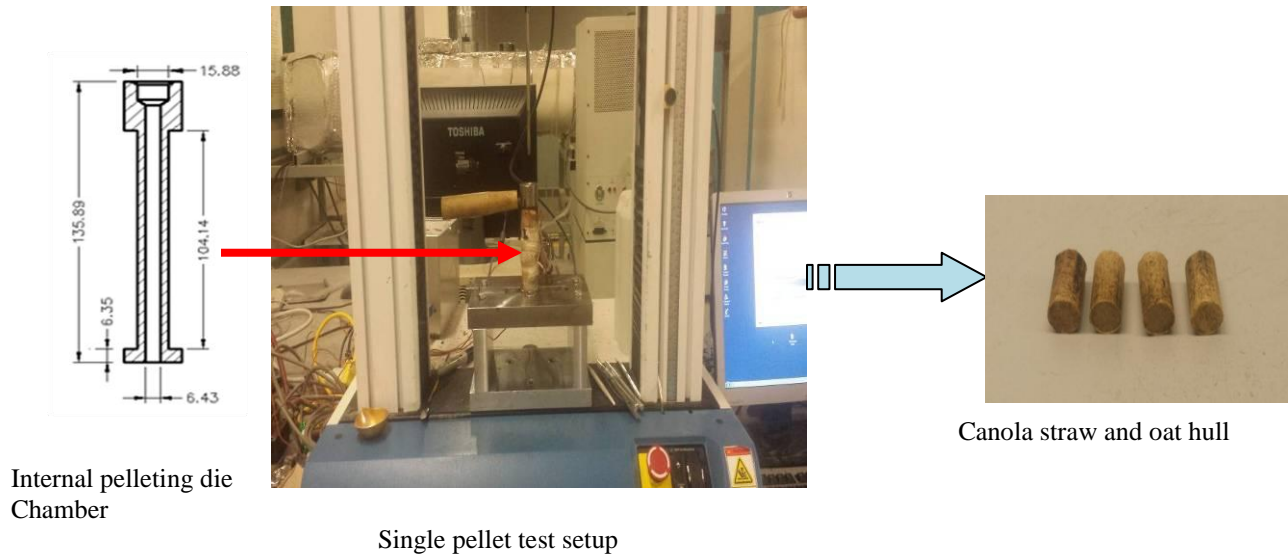
Figure 3.4. Internal sectional view of the single pelleting die unit (dimensions in mm) fixed in the Instron universal tester.

3.3.10. Pellet Density and Dimensional Stability

The height, diameter, and mass of pelleted samples from microwave-assisted alkali pretreated and untreated straws were measured immediately after pelleting using digital calipers to calculate the volume and pellet density of the samples. The pellets were stored in Ziploc plastic bags at room temperature at both stages for further analysis. After two weeks, the diameter, height, and mass of the pelleted samples were measured to calculate the dimensional stability of the pellets (Kashaninejad and Tabil 2011).

$$\text{Dimensional stability} = \frac{Vol_{14} - Vol_0}{Vol_0} \times 100\% \qquad (3.4)$$

Vol_0 = volume of pellets immediately after pelleting (mm^3); Vol_{14} = volume of pellets 14 days after pelleting (mm^3).

3.3.11. Tensile Strength Test

The diametral compression test as reported by Tabil and Sokhansanj (1997) and Kashaninejad et al (2010) was used to determine the tensile strength of microwave-assisted alkali pretreated and untreated canola straw and oat hull pellets. The pellets were cut diametrally into specimens of thickness of approximately 2.5 mm using a laser cutting machine. The single cut pellet was placed at the middle of a padded platen fastened to an Instron Tm machine (Figure 3.5) and compressed with the upper plunger until failure occurred. The Instron was fitted with a 5000 N load cell and the samples were compressed at a crosshead speed of 1 mm/min. The specimen fractured cracking into halves and failure occurred along the axis (Kashaninejad and Tabil 2011; Fell and Newton 1968; Fell and Newton 1970). Thirteen replicates were made for each sample. The fracture force was recorded and the tensile strength calculated as:

$$\delta_x = \frac{2F}{\pi d l} \qquad\qquad (3.5)$$

where δ_x is tensile strength (horizontal) stress (Pa); F is load at fracture (N); d is specimen diameter (m) and l is specimen thickness (m).
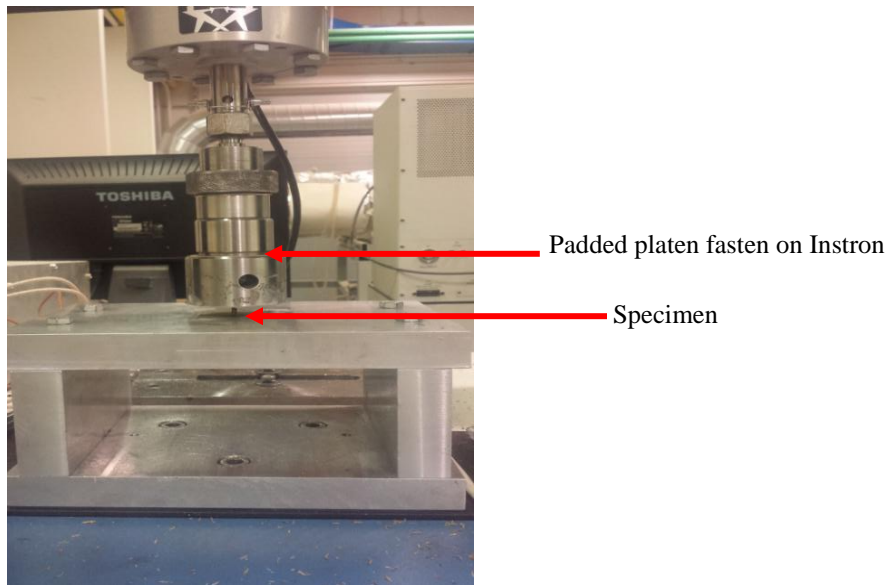
Figure 3.5. Instron universal tester fixed with padded platen used for tensile strength testing.

3.3.12 Statistical Analysis

Response surface methodology (RSM) is a statistical technique for designing experiments, building models, evaluating effects of factors which extract maximal information with minimal number of runs (Yue et al. 2008; Ma et al. 2009). In order to statistically study the effect of microwave treatment and alkali solution, a user-defined design (UDD) was applied via analysis of variance (ANOVA) to investigate the effect of microwave heating time and alkali concentration on compaction of canola straw and oat hull. The range and levels of variables determined are shown in Table 3.1 and a polynomial quadratic equation was fitted to the generated data to evaluate the effect of each independent variable on the responses:

$$y_n = \beta_0 + \sum_{i=1}^{2} \beta_i x_i + \sum_{i=1}^{2} \beta_{ii} x_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{2} \beta_{ij} x_i x_j \quad (n = 1, 2, 3, 4, 5, 6) \qquad (3.6)$$

where x_1 the alkali concentration (%) and x_2 the microwave heating time (min) are the independent variables which influence the response variables y (pellets density (kg/m^3), dimensional stability (%), tensile strength (MPa), ash content (%), bulk density (kg/m^3), particle density (kg/m^3); β_0 the offset term, β_i is the í linear term, β_{ii} is the quadratic term and β_{ij} is the íj interaction terms in the equation. The response surfaces of the variables in the experimental design domain were analyzed using Design Expert software (Karber 2013).

Table 3.1. Code levels for independent variables used in the UDD and actual factor levels corresponding to coded factor levels.

Independent variable	Code	Actual factor level at coded factor levels		
		-1	0	1
A - Alkali conc. (%)	X_1	0	0.75	1.5
B - MW time (min)	X_2	6	12	18

3.4 Results and Discussion

3.4.1 Physical Properties

Table 3.2 shows the physical properties of ground canola straw and oat hulls. The geometric mean particle diameter of canola straw was slightly smaller than that of oat hull samples. The ash content was higher in canola straw samples compared to oat hull samples. This may be variation in moisture content and mechanical properties of the different biomass. The canola straw ground in the hammer mill using 1.6 mm screen size was the finest among the ground biomass (Appendix A). However, the oat hull sample ground in the hammer mill with 1.6 mm screen had the highest bulk and particle densities 331.32 and 1440.51 kg/m^3, respectively. Samples ground in the hammer mill using a large screen size, e.g. 3.2 mm, resulted in lower bulk and particle densities.

Table 3.2. Physical properties of ground canola straw and oat hull

Sample	Screen size (mm)	Moisture content as received[a] (% wb)	Moisture content ground sample[a] (% wb)	d^a_{gw} (mm)	S^a_{gw} (mm)	Ash content[a] (%)	Bulk density[a] (kg/m^3)	Particle density[a] (kg/m^3)
Canola straw	1.6	9.08 ± 0.45	7.64 ± 0.59	0.348 ± 0.02	0.280 ± 0.01	6.47 ± 0.87	168.14 ± 2.67	1305.53 ± 46.08
	3.2		8.28 ± 0.39	0.520 ± 0.04	0.498 ± 0.01	6.66 ± 1.56	141.16 ± 2.10	1220.41 ± 6.20
Oat hull	1.6	9.72 ± 0.15	6.96 ± 0.33	0.370 ± 0.00	0.217 ± 0.01	5.31 ± 0.26	331.32 ± 4.39	1440.51 ± 3.25
	3.2		7.7 ± 0.12	0.547 ± 0.00	0.284 ± 0.00	5.65 ± 1.62	285.10 ± 9.16	1391.01 ± 8.40

Geometric mean diameter = d_{gw}; Geometric standard deviation = S_{gw}.
[a] Mean ± standard deviation of three replicates.
[b] Mean ± standard deviation of five replicates

Table 3.3 and 3.4 show the physical properties of microwave-assisted alkali pretreated canola straw (CS) and oat hull (OH), respectively. It was observed that samples pretreated with microwave alone showed lower bulk and particle densities 108.10 kg/m^3 and 982.42 kg/m^3, respectively, than the untreated samples (Table 3. 2). The highest bulk densities was observed in microwave-assisted alkali pretreated oat hull (336.55 kg/m^3) and canola straw (260.11 kg/m^3), whereas the highest particle densities was observed in canola straw (1572.56 kg/m^3) and oat hull (1559.22 kg/m^3). Increasing the time and alkali concentration increased the ash content and bulk density of microwave-alkali pretreated canola straw and oat hull. The analysis of variance of the

data shows that microwave heating time and alkali concentration significantly affected the bulk density of microwave-alkali pretreated canola straw and ash content of microwave-alkali pretreated oat hull in treatments NaOH and KOH/1.6 and 3.2 mm, and microwave heating time had a significant effect on the bulk density of microwave-alkali pretreated oat hull. Similarly, increasing the alkali concentration increased the particle density for microwave-alkali pretreated canola straw and oat hull except at 3.2 mm 0.75% NaOH. The microwave heating time did not show a significant effect on particle density for microwave-alkali pretreated oat hull and canola straw. The significant effects of alkali concentration and microwave heating time in the pretreated samples were as a result of microwave pretreatment which caused the swelling of material and increased the internal surface area of lignocellulosic biomass structure. Similar result was reported by Kashaninejad and Tabil (2011). The ANOVA tables are presented in the appendix B and C. Canola straw and oat hull pretreated by microwave-assisted alkali showed higher bulk and particle densities than untreated samples. Kashaninejad and Tabil (2011) reported that this is a result of increased depolymerized components and ash content of pretreated samples. In addition, samples pretreated with microwave/NaOH had higher bulk and particle densities than samples pretreated with microwave/KOH.

Table 3.3. Average ash content and bulk and particle densities of microwave-assisted alkali pretreated biomass samples.

Sample	Screen size (mm)	Alkali	Concentration (%)	MW heating time (min)	Microwave pretreated temperature[b] (°C)	Ash content[a] (%)	Bulk density[b] (kg/m^3)	Particle density[b] (kg/m^3)
			0	6	92.20 ± 0.10	5.16 ± 0.29	122.43 ± 1.43	1262.91 ± 30.54
			0	12	92.33 ± 0.15	5.33 ± 0.57	134.57 ± 1.77	1206.60 ± 7.39
			0	18	112.93 ±7.39	5.48 ± 0.48	137.72 ± 1.85	1124.45 ± 8.44
			0.75	6	72.82 ± 4.79	15.50 ± 0.50	149.41 ± 0.22	1514.18 ± 12.19
			0.75	12	75.24 ± 0.69	14.83 ± 0.28	171.54 ± 1.53	1423.10 ± 10.48
		NaOH	0.75	18	82.88 ± 8.70	14.33 ± 0.28	183.15 ± 2.29	1303.16 ± 5.36
	1.6		1.5	6	75.16 ± 1.33	20.13 ± 1.02	160.22 ± 2.48	1572.56 ± 81.84
			1.5	12	77.20 ± 1.36	22.17 ± 0.57	194.72 ± 2.22	1472.80 ± 2.98
			1.5	18	78.90 ± 1.10	22.96 ± 0.07	260.11 ± 0.90	1358.89 ± 5.27
			0.75	6	76.62 ± 2.61	12.83 ± 0.58	137.79 ± 0.89	1389.39 ± 8.86
			0.75	12	76.76 ± 1.55	12.67 ± 0.28	154.45 ± 1.55	1428.87 ± 13.54
		KOH	0.75	18	78.72 ± 1.65	12.33 ± 0.28	157.00 ± 0.73	1134.54 ± 9.08
			1.5	6	90.30 ± 1.33	19.33 ± 0.28	145.33 ± 0.75	1411.14 ± 3.02
Canola			1.5	12	91.76 ± 0.24	19.67 ± 0.57	173.98 ± 2.46	1496.22 ± 8.69
			1.5	18	116.92 ± 3.38	19.83 ± 0.29	200.99 ± 2.06	1343.62 ± 7.44
straw			0	6	92.07 ± 0.23	5.17 ± 0.28	108.10 ± 1.52	1033.48 ± 11.03
			0	12	92.23 ± 0.06	5.33 ± 0.28	116.40 ± 1.09	1045.16 ± 16.35
			0	18	116.67 ± 10.47	5.50 ± 0.50	126.96 ± 3.56	982.42 ± 20.54
			0.75	6	91.60 ± 0.10	15.17 ± 0.29	131.71 ± 3.05	1324.92 ± 5.20
			0.75	12	92.04 ± 0.36	14.67 ± 0.29	148.11 ± 1.83	1423.39 ± 18.12
		NaOH	0.75	18	119.02 ± 8.19	14.33 ± 0.58	170.48 ± 2.18	1229.76 ± 20.79
	3.2		1.5	6	90.36 ± 0.90	21.17 ± 0.58	153.09 ± 2.68	1462.90 ± 2.73
			1.5	12	91.90 ± 0.21	22.33 ± 0.28	182.61 ± 3.74	1466.03 ± 4.01
			1.5	18	123.26 ± 8.18	22.50 ± 1.00	247.76 ± 3.24	1297.84 ± 19.89
			0.75	6	91.24 ± 0.09	13.17 ± 0.28	114.86 ± 3.16	1285.89 ± 11.96
			0.75	12	91.38 ± 0.11	13.00 ±0.00	133.13 ± 2.98	1335.35 ± 9.93
		KOH	0.75	18	124.32 ± 6.98	12.67 ± 0.58	137.40 ± 1.70	1043.47 ± 5.22
			1.5	6	91.04 ± 0.08	20.17 ± 0.76	123.82 ± 1.12	1429.45 ± 6.89
			1.5	12	91.42 ± 0.24	20.50 ± 0.50	164.70 ± 3.58	1511.66 ± 8.47
			1.5	18	124.12 ± 5.01	20.67 ± 0.29	190.07 ± 1.94	1281.60 ± 1.84

[a] Mean ± standard deviation of three replicates;
[b] Mean ± standard deviation of five replicates.

Table 3.4. Average ash content and bulk and particle densities of microwave-assisted alkali pretreated biomass samples.

Sample	Screen size (mm)	Alkali	Concentration (%)	Microwave heating time (min)	Microwave pretreated temperature[b] (°C)	Ash content[a] (%)	Bulk density[b] (kg/m³)	Particle density[b] (kg/m³)
			0	6	92.56 ± 0.29	4.67 ± 0.28	256.60 ± 2.53	1427.75 ± 1.90
			0	12	93.03 ± 0.23	4.83 ± 0.58	264.84 ± 2.23	1430.20 ± 3.24
			0	18	93.86 ± 0.10	5.00 ± 0.50	321.27 ± 3.47	1410.10 ± 3.43
		NaOH	0.75	6	90.78 ±4.02	8.50 ± 0.50	235.95 ± 2.10	1465.14 ± 5.45
			0.75	12	92.26 ± 0.27	9.67 ± 0.29	270.10 ± 3.45	1502.91 ± 3.28
			0.75	18	106.62 ± 3.75	9.83 ± 1.26	334.46 ± 1.99	1502.89 ± 3.04
			1.5	6	88.45 ± 5.73	15.17 ± 0.29	280.39 ± 1.22	1544.32 ± 2.47
	1.6		1.5	12	92.75 ± 0.07	15.83 ± 0.28	329.28 ± 3.70	1557.82 ± 2.86
			1.5	18	104.55 ± 8.83	16.17 ± 0.28	353.11 ± 4.58	1548.69 ± 1.87
		KOH	0.75	6	92.87 ± 0.06	7.00 ± 0.00	243.14 ± 2.69	1447.42 ± 20.92
			0.75	12	92.90 ± 0.10	7.17 ± 0.76	276.28 ± 2.57	1451.40 ± 5.83
			0.75	18	111.97 ± 2.60	7.83 ± 1.04	298.96 ± 2.57	1464.83 ± 4.13
			1.5	6	92.44 ± 0.18	13.00 ± 0.50	247.12 ± 4.35	1498.86 ± 4.61
			1.5	12	92.58 ± 0.28	13.17 ± 0.28	290.26 ± 6.56	1546.26 ± 3.00
Oat hull			1.5	18	114.88 ± 8.18	13.50 ± 0.87	339.04 ± 5.50	1523.19 ± 3.33
			0	6	92.53 ± 0.23	4.50 ± 0.00	207.07 ± 3.56	1373.74 ± 4.94
			0	12	92.50 ± 0.26	4.67 ± 0.28	206.46 ± 2.41	1361.42 ± 3.36
			0	18	95.00 ± 2.18	5.33 ± 0.76	240.53 ± 1.46	1394.83 ± 2.81
		NaOH	0.75	6	92.00 ± 0.20	8.83 ± 0.28	207.31 ± 1.58	1435.75 ± 6.46
			0.75	12	92.30 ± 0.36	9.00 ± 0.50	238.94 ± 5.06	1506.29 ± 2.20
			0.75	18	126.30 ± 5.98	9.50 ± 0.50	253.98 ± 4.10	1505.66 ± 3.12
	3.2		1.5	6	92.38 ± 0.22	15.00 ± 0.50	236.56 ± 3.52	1533.35 ± 3.15
			1.5	12	92.68 ± 0.59	15.67 ± 0.76	336.55 ± 2.58	1559.22 ± 1.24
			1.5	18	119.43 ± 8.42	16.00 ± 0.50	283.27 ± 4.70	1548.87 ± 1.62
		KOH	0.75	6	92.73 ± 0.24	7.17 ± 0.76	209.05 ± 3.59	1456.67 ± 2.93
			0.75	12	92.40 ± 0.18	7.50 ± 0.50	217.53 ± 2.53	1464.51 ± 2.08
			0.75	18	120.70 ± 4.24	8.00 ± 0.50	244.91 ± 3.91	1457.28 ± 3.67
			1.5	6	92.60 ± 0.36	13.33 ± 0.28	221.49 ± 4.73	1507.52 ± 4.02
			1.5	12	93.27 ± 0.06	13.67 ± 0.58	257.88 ± 2.77	1541.46 ± 1.47
			1.5	18	115.70 ± 3.40	13.83 ± 0.29	258.75 ± 5.25	1530.87 ± 2.55

[a] Mean ± standard deviation of three replicates;
[b] Mean ± standard deviation of five replicates.

3.4.2 Chemical Composition of Microwave-Assisted Alkali Pretreated Canola Straw and Oat Hull.

The composition of canola straw was determined to be 42.39% cellulose, 16.41% hemicellulose and 14.15% lignin (Adapa et al. 2009), whereas oat hull had a composition of 34.61% cellulose, 29.80% hemicellulose and 10.01% lignin (Grewal et al. 2015). Table 3.5 shows the lignocellulosic composition of microwave-assisted alkali pretreated and microwave alone pretreated canola straw and oat hull samples. Reports from previous studies stated that alkali treatments dissolves lignin and hemicellulose, and microwave heating enhance breakdown of these components in alkali solutions (Kashaninejad and Tabil 2011; Kumar et al. 2009). The cellulose content increased with increasing alkali concentration and microwave heating time whereas the lignin content decreased with increase in microwave heating time and alkali concentration. This implies that there is a breakdown of the biomass matrix in the lignin and creates accessibility and digestibility of cellulose and hemicellulose (Kumar et al. 2009; Taherzadeh and Karimi 2008). The lignin content of pretreated canola straw and oat hull samples was lower than microwave alone pretreated samples except in treatment CS 1.6 mm/0.75% KOH 12 min. The lignin decrease indicates solubilization in the alkaline aqueous solution and increase in cellulose was as a result of solubilization from other components in the alkali solution. Also, increase in cellulose content by microwave heating was facilitated by dissolution of components in alkaline solutions (Zhu et al. 2005; Zhu et al. 2006). The microwave-assisted alkali pretreatment removed more hemicellulose and lignin in canola straw than oat hull samples. In addition, microwave-alkali in both feedstocks resulted in higher solubilization of cellulose and, decrease in hemicellulose and lignin. The stronger alkaline pretreatment in combination with long microwave heating time caused more solubilization of cellulose, hemicellulose and lignin. Zhu et al. (2006a and 2005) reported a similar result with wheat straw and rice straw. For the both feedstocks in this study, canola straw samples showed higher solubilization with the alkali solution than the oat hull in microwave-assisted alkali pretreatment. This shows that alkaline used in the pretreatments caused swelling and lignin structure disruption in the biomass that resulted to solubility of lignin in the samples (Tomas-Pejo et al. 2011; Taherzadeh and Karimi 2008). Kashaninejad and Tabil (2011) reported that the main aim of using alkali solution during microwave pretreatment method is to disintegrate the ester bonds between lignin and carbohydrate in the biomass and this statement is supported with the data presented herein.

Table 3.5 Chemical composition (% dry basis) of microwave-assisted alkali pretreated canola straw and oat hull.

Sample	Screen size (mm)	Alkali	Concentration (%)	Microwave heating time (min)	C[a] (%)	H[a] (%)	L[a] (%)
Canola straw	1.6		0	18	63.1 ± 32.0	5.5 ± 6.3	5.0 ± 0.8
		NaOH	1.5	18	59.1 ± 0.5	9.4 ± 8.3	4.3 ± 1.2
			1.5	6	37.8 ± 3.1	7.2 ± 6.5	4.7 ± 0.6
		KOH	0.75	12	53.6 ± 9.2	10.6 ± 9.2	5.8 ± 0.3
			1.5	6	56.9 ± 17.0	7.7 ± 9.0	4.6 ± 0.5
	3.2		0	18	60.0 ± 21.8	6.2 ± 5.5	5.6 ± 1.0
		NaOH	0.75	12	54.2 ± 2.3	6.7 ± 5.8	5.1 ± 0.6
			0.75	6	38.2 ± 2.7	8.7 ± 7.5	5.3 ± 0.3
		KOH	0.75	12	30.8 ± 2.9	13.8 ± 13.0	5.0 ± 1.6
			1.5	6	63.4 ± 35.0	10.3 ± 9.2	4.4 ± 0.5
Oat hull	1.6		0	12	36.7 ± 17.0	10.5 ± 9.1	7.5 ± 1.2
		NaOH	0.75	18	42.8 ± 11.3	15.6 ± 13.8	6.3 ± 1.0
			1.5	18	37.1 ± 8.5	14.3 ± 12.6	4.2 ± 1.2
		KOH	1.5	18	56.4 ± 17.9	16.0 ± 13.8	4.8 ± 0.9
			1.5	6	41.8 ± 14.0	12.9 ± 11.5	5.7 ± 1.6
	3.2		0	6	51.2 ± 19.5	11.0 ± 9.6	9.2 ± 0.4
		NaOH	0.75	6	22.7 ± 11.0	12.9 ± 14.4	6.8 ± 2.2
			1.5	18	48.7 ± 8.3	14.4 ± 13.3	5.1 ± 0.8
		KOH	0.75	12	47.9 ± 18.2	16.0 ± 16.0	5.4 ± 0.6
			1.5	18	62.6 ± 2.0	10.4 ± 18.0	6.4 ± 1.3

C – cellulose, H – hemicellulose, L – lignin;
[a] Mean ± standard deviation of three replicates.

3.4.3 Pellet Density

Table 3.6 and 3.7 show the effect of microwave-alkali pretreatments on pellet density, dimensional stability and tensile strength for canola straw and oat hull pellets. The surface of microwave-alkali pretreated samples appeared smoother and darker than alkali treated and untreated samples, and Kashaninejad and Tabil (2011) reported similar result with pellets produced from wheat and barley straw grinds. The microwave-assisted alkali pretreated samples showed the highest pellet density (canola straw 1392.21 kg/m^3 and oat hulls 1292.59 kg/m^3) compared to microwave alone and untreated samples. Increasing the alkali concentration increased the pellet density of the samples. Increasing the microwave heating time decreased the

pellet density of canola straw samples with treatments of 1.6 mm/0, 0.75 and 1.5% KOH and 3.2 mm/ 0 and 0.75%; for oat hull, the microwave heating time increased pellet density in treatments of 1.6 mm/1.5% NaOH, 0.75 and 1.5% KOH and decreased in treatment 3.2mm/0.75% KOH. Iroba et al. (2014) reported that samples release binding agent (lignin) which increase the adhesion within the particles, activate the intermolecular bonds within the contact area of the samples, and improves the mechanical interlocking of the particles during pelleting.

Analysis of variance of the data shows that alkali concentration significantly ($P < 0.05$) affected canola straw and oat hull pellet density. Microwave heating time had a significant effect for samples with treatments of KOH/1.6 mm for canola straw and oat hull pellets. Microwave/NaOH pretreatment was more effective at the initial heating time for 0.75% alkali concentration in increasing the initial density of the pellets while microwave/KOH pretreatment was more effective at 1.5% alkali concentration in increasing the initial pellet density (Appendix D and E).

3.4.4 Dimensional stability

The dimensional stability values for canola straw and oat hull pellets are presented in Table 3.6 and 3.7. Samples pretreated with microwave-assisted alkali have the highest dimensional stability (close to 0) as compared to samples pretreated with microwave heating only and untreated samples. In canola straw, microwave-assisted alkali pretreated canola straw and oat hull pellets had the highest dimensional stability of 3.2 mm/0.75% NaOH and KOH at 6 min, respectively. This is because samples released the binding agent (lignin) which increased the adhesion within the particles, activated the intermolecular bonds within the contact area of the samples and in addition enhanced the mechanical interlocking of the particles (Iroba et al. 2014). The data indicated that dimensional stability of canola straw pellets decreased with increasing alkali concentration in treatments 6 and 18 min/NaOH 1.6 mm screen size; 12 and 18 min/NaOH and KOH 3.2 mm screen size. Oat hull pellet dimensional stability decreased with increasing alkali concentration in treatments 18 min/NaOH; 6 and 18 min/KOH 1.6 mm screen size; 12 and 18 min/NaOH and 18 min/KOH 3.2 mm screen size. Lower microwave heating time resulted in higher stability of the canola straw pellets for treatment combination of: 1.6 mm/0; 3.2 mm/0 and 1.5% KOH and in oat hulls pellets 1.6 mm/0.75 and 1.5% KOH, and 3.2 mm/ 0,

0.75 and 1.5% NaOH and KOH. Iroba et al. (2014) and Tabil (1996) reported that when biomass is heated, the lignin becomes soft, melts and exhibits thermosetting binder resin properties to produce pellets with higher density and dimensional stability.

Analysis of variance shows that alkali concentration and microwave heating time significantly ($P < 0.05$) affected the dimensional stability of the canola straw and oat hull pellets in 3.2 mm screen size for both NaOH and KOH and 1.6 mm canola straw. In the other treatments, only microwave heating time showed significant effect on the pellet stability of oat hull KOH/3.2 mm. From the analysis of variance, both alkali concentration and microwave heating time affected the dimensional stability of microwave-assisted alkali pretreated canola straw and oat hull pellets (Appendix D and E). Pellets produced from microwave-alkali pretreated samples will present easy handling and storage and result in efficient transportation in terms of withstanding shear, impact, rotation and tumbling with minimal generation of fine particulate matter (Iroba et al. 2014; Adapa et al. 2009; Mani et al. 2006).

3.4.5 Tensile Strength of Pellets

Table 3.6 and 3.7 show the tensile strength (evaluated using equation (3.5)) and the fracture load values of the pellets produced from microwave-assisted alkali pretreated, microwave alone and untreated canola straw and oat hull which were evaluated using equation (3.5). The observed data indicates that alkali concentration and microwave heating time are important factors and process condition for the physical characteristics of the pellets. Microwave-assisted-alkali pretreated pellet samples showed highest tensile strength (canola straw =5.22 MPa at 1.6 mm 1.5% NaOH 6 min and oat hull =3.36 MPa at 1.6 mm 1.5% NaOH 18 min). Increasing alkali concentrations increased the tensile strength of canola straw and oat hull pellets. This means that microwave-assisted alkali pretreatment has the ability to disintegrate the structure of lignocellulosic biomass involved in particle binding (Kashaninejad and Tabil 2011) and results in the breakdown of lignin components. Thus, the lignin after pretreatment assisted in the particle binding mechanisms during pelleting resulting in pellets with higher tensile strength and fracture load (Iroba et al. 2014). Longer microwave heating time resulted in lower tensile strength of canola straw pellets but higher tensile strength of oat hull pellets in treatments combinations of: 1.6 mm/ 1.5% NaOH and KOH; 3.2 mm/ 0, 1.5% NaOH and, 0.75;

1.5% KOH. Samples pretreated with microwave alone (MW/distilled water) had lower tensile strength and fracture load than others. This is because water and heat along are not sufficient in disintegrating the lignocellulosic matrix of biomass (Iroba et al. 2014).

Analysis of variance performed on the data shows that alkali the concentration and the microwave heating time had significant effects ($P < 0.05$) on the tensile strength of canola straw pellet in treatments NaOH and KOH/1.6 mm and 3.2 mm KOH/3.2 mm, and oat hull pellets in treatments 3.2 mm NaOH/3.2 mm. Consequently, only alkali concentration had significant effect on the tensile strength other treatments of canola straw and oat hull pellets (Appendix D and E).

The representative 3D response surface and the 2D contour plots of the responses from microwave-assisted alkali pretreated canola straw and oat hull pellets are shown (Appendix F and G).In order to depict the interactive effects, each of these responses pellet density, dimensional stability and tensile strength were kept constant while the two independent variables (alkali concentration and MW heating) varied in certain ranges. The response surfaces and contour plots of the microwave heating time differed with the alkali solution used in the study. Comparatively, notable interactions among the variables were shown in microwave/NaOH pretreated samples by their shapes and contours compared to KOH pretreated samples. The interaction among the pellet density, dimensional stability and tensile strength significantly influenced the pellet quality of the samples regardless of the alkaline concentration and microwave heating time of the samples.

Furthermore, high alkali concentration with long microwave heating resulted in high pellet density in canola straw and oat hull pellets. However, less negligible interactions were shown with dimensional stability of the samples pellets by the irregular shape nature of the contour plots (1.6 mm CS and OH/ 3.2mm OH KOH), while comparatively prominent interactions were shown with pellets densities and tensile strengths by the rectangular curved nature of the contour plots of the samples. In other words, the interaction effects of alkali concentration and microwave heating time significantly affected the physical qualities of canola straw and oat hull pellets.

Table 3.6. Effect of MW/Alkali pretreatments on pellet density, dimensional stability and tensile strength for canola straw pellets.

Sample	Screen size (mm)	Alkali	Concentration (%)	Microwave heating time (min)	Pellets density[a] (kg/m³)	Dimensional stability[a] (%)	Tensile strength[ab] (MPa)
Untreated canola straw	1.6	-	-	-	1030.87 ± 9.89	3.95 ± 1.59	0.26 ± 0.09
	3.2	-	-	-	1060.82 ± 12. 99	5.23 ± 0.87	0.62 ± 0.27
Canola straw	1.6		0	6	1066.17 ± 28.38	0.51 ± 2.94	0.72 ± 0.31
			0	12	1037.10 ± 8.95	1.43 ± 1.25	0.74 ± 0.19
			0	18	1021.65 ± 7.92	3.27 ± 0.86	0.56 ± 0.15
		NaOH	0.75	6	1286.59 ± 14.34	0.05 ± 0.94	4.71 ± 0.74
			0.75	12	1309.35 ± 7.15	2.21 ± 0.52	2.66 ± 0.52
			0.75	18	1248.24 ± 9.12	1.13 ± 0.70	1.79 ± 0.28
			1.5	6	1319.21 ± 11.09	0.76 ± 0.91	5.22 ± 1.21
			1.5	12	1327.98 ± 6.72	0.78 ± 0.32	3.44 ± 1.02
			1.5	18	1370.27 ± 12.62	0.79 ± 0.62	2.31 ± 0.48
		KOH	0.75	6	1243.01 ± 8.53	0.04 ± 0.52	2.67 ± 0.41
			0.75	12	1195.28 ± 8.25	0.17 ± 0.68	1.90 ± 0.34
			0.75	18	1160.16 ± 8.15	1.44 ± 0.51	0.85 ± 0.30
			1.5	6	1392.21 ± 10.74	0.83 ± 0.58	3.78 ± 0.62
			1.5	12	1339.64 ± 7.42	0.26 ± 0.39	2.58 ± 0.37
			1.5	18	1321.01 ± 17.10	1.63 ± 2.51	2.11 ± 0.47
Canola straw	3.2		0	6	1089.17 ± 19.24	0.16 ± 0.64	1.19 ± 0.44
			0	12	1086.86 ± 6.86	1.23 ± 0.69	1.04 ± 0.27
			0	18	1029.82 ± 6.51	3.98 ± 0.95	0.81 ± 0.40
		NaOH	0.75	6	1324.75 ± 9.68	2.60 ± 0.48	4.85 ± 0.99
			0.75	12	1283.60 ± 10.08	0.33 ± 0.47	2.53 ± 0.88
			0.75	18	1277.29 ± 12.02	1.85 ± 0.65	1.69 ± 0.42
			1.5	6	1351.61 ± 14.86	1.66 ± 0.36	4.20 ± 1.03
			1.5	12	1345.57 ± 8.66	0.17 ± 0.37	4.11 ± 1.53
			1.5	18	1388.30 ± 9.61	1.18 ± 0.44	2.59 ± 0.70
		KOH	0.75	6	1201.33 ± 5.62	0.70 ± 0.38	2.07 ± 0.55
			0.75	12	1220.50 ± 7.49	0.27 ± 0.62	1.73 ± 0.35
			0.75	18	1176.32 ± 8.63	2.10 ± 0.90	1.41 ± 0.39
			1.5	6	1382.62 ± 5.73	0.83 ± 0.53	5.19 ± 0.60
			1.5	12	1344.09 ± 8.06	0.29 ± 0.49	3.19 ± 0.90
			1.5	18	1355.93 ± 11.53	1.38 ± 0.77	2.89 ± 0.70

[a] Mean ± standard deviation of ten pellets produced.
[b] Mean ± standard deviation of thirteen specimens tables made.

Table 3.7. Effect of MW/Alkali pretreatments on pellet density, dimensional stability and tensile strength for oat hull pellets.

Sample	Screen size (mm)	Alkali	Concentration (%)	Microwave heating time (min)	Pellets density[a] (kg/m³)	Dimensional stability[a] (%)	Tensile strength[ab] (MPa)
Untreated oat hull	1.6				1031.23 ± 35.64	7.75 ± 3.26	0.04 ± 0.03
	3.2				1087.74 ± 13.16	6.14 ± 1.93	0.39 ± 0.27
Oat hull	1.6		0	6	989.14 ± 22.44	3.39 ± 1.48	0.14 ± 0.14
			0	12	1029.53 ± 12.57	9.34 ± 1.29	0.04 ± 0.02
			0	18	1028.72 ± 15.64	5.76 ± 6.05	0.30 ± 0.27
		NaOH	0.75	6	1238.12 ± 13.72	0.84 ± 0.42	1.34 ± 0.42
			0.75	12	1209.12 ± 13.71	1.38 ± 0.82	1.58 ± 0.75
			0.75	18	1221.99 ± 11.63	5.28 ± 1.08	1.33 ± 0.91
			1.5	6	1198.89 ± 16.53	0.53 ± 1.15	1.19 ± 0.64
			1.5	12	1286.52 ± 5.62	0.70 ± 0.49	1.96 ± 1.51
			1.5	18	1292.59 ± 30.61	4.54 ± 1.08	3.36 ± 1.63
		KOH	0.75	6	1123.85 ± 9.12	0.53 ± 0.67	0.57 ± 0.29
			0.75	12	1164.37 ± 9.76	0.34 ± 0.73	0.82 ± 0.44
			0.75	18	1166.59 ± 17.28	4.79 ± 1.22	0.73 ± 0.34
			1.5	6	1185.69 ± 24.27	1.04 ± 0.84	0.63 ± 0.31
			1.5	12	1220.42 ± 8.28	1.45 ± 0.65	0.83 ± 0.37
			1.5	18	1290.75 ± 18.82	3.30 ± 1.60	1.43 ± 0.65
Oat hull	3.2		0	6	1045.82 ± 9.10	0.44 ± 0.58	0.25 ± 0.16
			0	12	1018.03 ± 23.14	4.67 ± 1.44	0.30 ± 0.26
			0	18	1066.38 ± 11.81	11.78 ± 1.88	0.45 ± 0.29
		NaOH	0.75	6	1205.73 ± 11.65	1.68 ± 0.58	1.23 ± 0.68
			0.75	12	1198.83 ± 7.30	1.82 ± 0.87	1.17 ± 0.71
			0.75	18	1219.29 ± 8.54	6.56 ± 0.86	1.91 ± 1.37
			1.5	6	1218.86 ± 32.75	1.16 ± 2.31	1.28 ± 0.54
			1.5	12	1321.34 ± 8.33	1.07 ± 0.97	2.27 ± 1.67
			1.5	18	1274.09 ± 13.01	6.15 ± 0.76	2.65 ± 1.18
		KOH	0.75	6	1073.31 ± 7.69	1.15 ± 0.75	0.46 ± 0.28
			0.75	12	1160.83 ± 8.34	6.22 ± 1.38	0.87 ± 0.95
			0.75	18	1143.75 ± 8.74	7.66 ± 1.12	0.90 ± 0.53
			1.5	6	1212.34 ± 6.39	0.83 ± 0.67	1.00 ± 0.64
			1.5	12	1248.13 ± 9.13	3.46 ± 0.69	1.08 ± 0.69
			1.5	18	1210.94 ± 21.26	5.95 ± 1.18	1.17 ± 0.76

[a] Mean ± standard deviation of ten pellets produced.
[b] Mean ± standard deviation of thirteen specimens tables made.

3.4.6 Variable Optimization

The optimal condition goals for microwave-assisted alkali pretreatment of canola straw and oat hull pellets were extracted from numerical optimization by Design Expert software. The response variables (pellet density and tensile strength) are to be maximized and dimensional stability is to be minimized. In considering the level of importance, tensile strength is the most important property due to the physical resistance of pellets to the forces in pellet handling and transportation. Dimensional stability is next, indicating less dust generation during handling and is followed by pellet density because high density is another desirable property in pellet handling. Alkali concentration and microwave heating time were placed in range as shown in Table 3.8.

Table 3.8. Goal for optimization of variables during the experimental pelletization of canola straw and oat hull.

Variable	Goal	Level of importance
Independent		
Alkali concentration (%)	In range (0 to 1.5)	
MW heating time (min)	In range (6 to 18)	
Dependent		
Pellet density (kg/m^{-3})	Maximize	3
Dimensional stability (%)	Minimize	2
Tensile strength (MPa)	Maximize	1

Table 3.9 presents the optimum operating parameters and desirability scores of all the variables as extracted by the software. The desirability function is a simultaneous determination of optimum settings of input variables that can determine the best optimum performance levels for one or more responses. Desirability functions are used in order to obtain qualitative and quantitative responses by the fast transformation of different responses to one measurement. Its general approach is to first convert the response into an individual desirability function (d_i) that varies from 0 (lowest desirability) to 1 (highest desirability) (Mourabet et. al 2012). The individual desirability values for each dependent variable can be computed using equation (3.7).

$$D = (d_1 \times d_2 \times \ldots \times d_n)^{\frac{1}{n}} = \left(\prod_{i=1}^{n} d_i\right)^{\frac{1}{n}} \qquad (3.7)$$

where, D is the overall desirability geometric mean of all the individual desirabilities (d_i) that range from 0 (least) to 1 (most); n is the number of responses being optimized (Karber 2013).

According to Table 3.9, the results showed that 1.5% alkali concentration was considered optimal regardless of the screen size of hammer mill used to grind the samples whereas reduced microwave heating time (approximately 6 min) was considered optimal for canola straw and longer microwave heating (9-18 min) for oat hull samples in both screen sizes of hammer mill used to grind the samples. At the same time, it was observed that optimum operating condition selected for microwave-assisted alkali pretreatment were best for canola straw and oat hull which were hammer milled with 1.6 mm screen size.

Table 3.9. Optimum conditions for producing canola straw and oat hull pellets under microwave-assisted alkali (NaOH and KOH) pretreatment.

Sample	Screen size (mm)	Alkali	Concentration (%)	Microwave heating time (min)	Pellets density (kg/m³)	Dimensional stability (%)	Tensile strength (MPa)	Desirability
Canola straw	1.6	NaOH	1.28	6.93	1330.99	1.02	4.93	0.867
	3.2		1.48	6	1352.12	2.02	4.79	0.931
	3.2	KOH	1.5	6	1367.46	0.46	4.86	0.937
	1.6		1.5	6.28	1391.20	0.19	3.75	0.963
Oat hull	1.6	NaOH	1.38	14.72	1285.22	1.79	2.41	0.789
	3.2		1.5	13.66	1282.49	2.36	2.20	0.796
	1.6	KOH	1.49	18	1276.29	3.40	1.33	0.797
	3.2		1.5	9.01	1226.16	1.66	1.03	0.851

3.5 Conclusions

Microwave-assisted alkali pretreatment was found to enhance the densification of canola straw and oat hull. The following conclusions can be drawn from this investigation:

1. Cellulose content increased with increasing alkali concentration and microwave heating time in microwave-assisted alkali pretreatment. Microwave-assisted alkali pretreatment removed more hemicellulose and lignin in canola straw than oat hull samples.

2. Ground samples pretreated by microwave-assisted alkali had significantly higher ash content, bulk and particle densities (especially biomass samples pretreated with microwave/NaOH) than samples pretreated with microwave heating only and untreated samples.

3. Biomass samples hammer milled with 1.6 mm screen size resulted in pellets with better physical quality compared to those samples hammer milled with 3.2 mm screen size.

4. Alkali concentration of 1.5% with a microwave power level 713 W and microwave heating time of approximately 6 min resulted in high tensile strength of canola straw pellets whereas a microwave heating time of 9-18 min and an alkali concentration of approximately 1.5% resulted in high tensile strength oat hull pellets.

5. Microwave/NaOH pretreatment resulted in better physical quality pellets from both canola straw and oat hull samples than microwave/KOH pretreatment.

3.6 REFERENCES

1. Adapa, P., L. Tabil and G. Schoenau. 2011. Grinding performance and physical properties of non-treated and steam exploded barley, canola, oat and wheat straw. *Biomass and Bioenergy* 35: 549 – 561.

2. Adapa, P.K., L.G. Tabil and G.J. Schoenau. 2013. Factors affecting the quality of biomass pellet for biofuel and energy analysis of pelleting process. *International Journal Agricultural and Biological Engineering* 6(2): 1 – 12.

3. Adapa, P., L. Tabil, G. Schoenau. 2009. Compaction characteristics of barley, canola, oat and wheat straw. *Biosystems Engineering* 104: 335 – 344.

4. Agbor, V.B., N. Cicek, R. Sparling, A. Berlin and B.D. Levin. 2011. Biomass pretreatment: Fundamentals toward application. *Biotechnology Advances* 29: 675-685.

5. Alvira, P., E. Tomas-Pejo, M. Ballesteros and M.J. Negro. 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. *Bioresource Technology* 101: 4851 – 4861.

6. Anna, C.S. and W. de Souza. 2012. Microscopy as a tool to follow deconstruction of lignocellulosic biomass. www.formatex.info/microscopy 5/book/639-645.pdf (2015/06/01).

7. ASABE. 2006. ASAE S358.2 - Moisture measurement – Forages. *ASABE Standards*. St. Joseph, MI: American Society of Agricultural and Biological Engineers.

8. ASABE. 2008. ASAE S319.4 (R2012) - Method of determining and expressing fineness of feed materials by sieve. *ASABE Standards*, St. Joseph, MI: American Society of Agricultural and Biological Engineers.

9. Azuma, J.I., F. Tanaka and T. Koshijima. 1984. Enhancement of enzymatic susceptibility of lignocellulosic wastes by microwave irradiation. *Journal of Fermentation Technology* 62(4): 377 – 384.

10. Balat, M., H. Balat and C. Oz. 2008. Progress in bioethanol processing. *Progress in Energy and Combustion Science* 34(5): 551 – 573.

11. Demirbas, A., M.F.M. Balat and H. Balat. 2009. Potential contribution of biomass to the sustainable energy development. *Energy Conversion and Management* 50 1746-1760.

12. Diaz, A.B., M.M. Moretti, C. Bezerra-Bussoli, C.daC.C Nunes, A. Blandino, R. da Silva and E. Gomes. 2015. Evaluation of microwave-assisted pretreatment of lignocellulosic biomass immersed in alkaline glycerol for fermentable sugars. *Bioresource Technology* 185: 316 – 323.

13. Fell, J.T. and J.M. Newton. 1968. The tensile strength of lactose tablets. *Journal of Pharmacy and Pharmacology* 20: 657 – 658.

14. Fell, J.T. and J.M. Newton. 1970. Determination of tablet strength by the diametral-compression test. *Journal of Pharmaceutical Science* 59(5): 688 – 691.

15. Gong, G., D. Liu and Y. Huang. 2010. Microwave-assisted organic acid pretreatment for enzymatic hydrolysis of rice straw. *Biosystems Engineering* 107: 67 – 73.

16. Grewal, R.K., M. Soleimani and L.G. Tabil. 2015. Investigations on biocomposites from oat hulls and biodegradable polymers. CSBE Paper No. 15-019. Winnipeg, Manitoba: CSBE/SCGAB.

17. Iroba, K.L and L.G. Tabil. 2013. Lignocellulosic biomass feedstock characteristics, pretreatment methods and pre-processing of biofuel and bioproduct applications. US and Canada perspectives. In *Biomass Processing, Conversion and Biorefinery*, ed. B. Zhang and Y. Wan, 61-98. New York, NY: Nova Science Inc.

18. Iroba, K.L., L.G. Tabil, S. Sokhansanj and V. Meda. 2014. Producing durable pellets from barley straw subjected to radio frequency-alkaline and steam explosion pretreatments. *International Journal of Agricultural and Biological Eng*ineering 7(3): 68 - 82.

19. Jenkins, B.M., L.L. Baxter and J. Koppejan. 2011. Biomass combustion. In *Thermochemical Processing of Biomass, Conversions into Fuels, Chemical and Power*, 1st ed.; R.C. Brown, 14 – 29. New York, NY: John Wiley and Sons.

20. Karber, S. 2013. Introduction to response surface methods. Part 1. Minneapolis, MN: Stat-Ease Design-Expert, Stat-Ease Incorporation.

21. Kashaninejad, M. and L. G. Tabil. 2011. Effect of microwave: chemical pretreatment on compression characteristics of biomass grinds. *Biosystems Engineering* 108: 36 – 45.

22. Kashaninejad, M., B. Kashaninejad and L.G. Tabil. 2010. Effect of microwave pretreatment on densification of wheat straw. ASABE Paper No. 1009051. St. Joseph, MI: ASABE.

23. Keshwani, D.R. and J.J. Cheng. 2010. Microwave-based alkali pretreatment of switchgrass and coastal bermudagrass for bioethanol production. *Biotechnology Progress* 26(3): 644 – 652.

24. Kumar, P., D.M. Barrett, M.J. Delwiche and P. Stroeve. 2009. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial and Engineering Chemistry Research* 48: 3713 – 3729.

25. Li, X., E. Mupondwa, S. Panigraphi, L.G. Tabil, S. Sokhansanj and M. Stumborg. 2012. A review of agricultural crop residue supply in Canada for Cellulosic ethanol production. *Renewable and Sustainable Energy Reviews* 16: 2954-2965.

26. Ma, H., W. Liu, X. Chen, Y. Wu and Z. Yu. 2009. Enhanced enzymatic saccharification of rice straw by microwave pretreatment. *Bioresource Technology* 100: 1279 – 1284.

27. Mani, S., L.G. Tabil and S. Sokhansanj. 2006. Effects of compressive force, particle size and moisture content on mechanical properties of biomass pellets from grasses. *Biomass and Bioenergy* 30: 648 – 654.

28. Mosier, N. S., C. Wyman, B. Dale, R. Elander, Y. Y. Lee, M. Holtzapple and M. R. Ladisch. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology* 96 (6): 673-686.

29. Mourabet, M., A. El Rhilassi, H. El Boujaady, M. Bennani-Ziatni, R. El Hamri and A. Taitai. 2012. Removal of fluoride from aqueous solution by adsorption on apatitic tricalcium phosphate using box-behnken design and desirability function. *Applied Surface Science* 258: 4402 – 4410.

30. Nomanbhay, S.M., R. Hussain and K. Palanisamy. 2013. Microwave-assisted alkaline pretreatment and microwave assisted enzymatic saccharification of oil palm empty fruit bunch fiber foe enhance fermentable sugar yield. *Journal of Sustainable Bioenergy System* 3: 7 – 17.

31. Oguocha, I.N.A. 2015. Materials characterization techniques. Department of Mechanical and Material Science Engineering, University of Saskatchewan, Saskatchewan, Canada.

32. Ooshima, H., K. Aso, Y. Harano and T. Yamamoto. 1984. Microwave treatment of cellulosic materials for their enzymatic hydrolysis. *Biotechnology Letters* 6(5): 289 – 294.

33. Quitain, A.T., M. Sasaki and M. Goto. 2013. Microwave-based pretreatment for efficient biomass-to-biofuel conversion. In *Pretreatment Techniques for Biofuels and Biorefineries*, ed. Z. Fang, 117 – 130. Berlin, Germany: Springer.

34. Quintero, J.A., L.E. Rincon and C.A. Cardona. 2011. Production of bioethanol from agro industrial residues as feedstocks. In *Biofuels: Alternative Feedstocks and Conversion Processes*, ed. A. Pandey, E. Gnansounou, C.G. Dussap, C. Larroche and S.C. Ricke, 253-260. Oxford UK: Academic Press.

35. Rodrigues, T. H. S., M.V.P. Rocha, G.R.D. Macedo and L.R.B. Goncalves, 2011. Ethanol production from cashew apple bagasse: improvement of enzymatic hydrolysis by microwave-assisted alkali pretreatment. *Applied Biochemistry and Biotechnology* 164: 929 – 943.

36. Saha, B. C., A. Biswas and M.A. Cotta. 2008. Microwave pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol. *Journal of Biobased Material and Bioenergy* 2: 210 – 217.

37. Sanchez, O.J and C.A. Cardona. 2008. Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresource Technology* 99(13): 5270 – 5295.

38. Saskatchewan Ministry of Agriculture field crop sheet. 2014. Field crop statistics. www.publications.gov.sk.ca/redirect.cfm?p=74877&i=83776 (2015/07/20).

39. Sluiter, A., B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton and D. Crocker. 2008. Determination of structural carbohydrates and lignin in biomass. NREL/TP-510-42618. Golden, CO: Office of Energy Efficiency and Renewable Energy, National Renewable Energy Laboratory.

40. Smith, D. 2013. Brief overview of biofuels. Reports 2 and 3. McGill University, Montreal, QC: BioFuelNet Canada.

41. Sun, Y. and J. Cheng. 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology* 83: 1 – 11.

42. Tabil L. G. and S. Sokhansanj. 1997. Bulk properties of alfalfa grind in relation to its compaction characteristics. *Applied Engineering in Agriculture* 13(4): 499 – 505.

43. Tabil, L.G. 1996. Binding and pelleting characteristics of alfalfa. Unpublished Ph.D. thesis. Saskatoon, Saskatchewan: Department of Agricultural and Bioresource Engineering, University of Saskatchewan.

44. Taherzadeh, M.J. and K. Karimi. 2008. Pretreatment of lignocellulosic waste to improve ethanol and biogas production: a review. *International Journal of Molecular Sciences* 9: 1621 – 1651.

45. Tomas-Pejo, E., P. Alvria, M. Ballesteros and M.J. Negro. 2011. Pretreatment technologies for lignocellulose to bioethanol conversion. In *Biofuels: Alternative Feedstocks and Conversion Processes,* ed. A. Pandey, E. Gnansounou, C.G. Dussap, C. Larroche and S.C. Ricke, 149 – 177. Oxford, UK: Academic Press.

46. Veal, M.W. 2010. Biomass logistics. In *Biomass to Renewable Energy Processes*, ed. J. Cheng, 129 – 130. New York, NY: CRC Press.

47. Xu, J. 2015. Microwave pretreatment. In *Pretreatment of Biomass: Processes and Technologies*, ed. A. Pandey, 157 – 172. Waltham, MA: Elsevier.

48. Yue, Z. B., H. Q. Yu, Z. H. Hu, H. Harada and Y. Y. Li. 2008. Surfactant-enhanced anaerobic acidogenesis of *Canna indica L.* by rumen cultures. *Bioresource Technology* 99: 3418 – 3423.

49. Zhao, Y., Y. Wang, J. Y. Zhu, A. Ragauskas and Y. Deng. 2008. Enhanced enzymatic hydrolysis of spruce by alkaline pretreatment at low temperature. *Biotechnology and Bioengineering* 99(6): 1320 – 1328.

50. Zhu, S., Y. Wu, Z. Yu, Q. Chen, G. Wu, F. Yu, C. Wang and S. Jin. 2006(a). Microwave-assisted alkali pre-treatment of wheat straw and its enzymatic hydrolysis. *Biosystems Engineering* 94(3): 437 – 442.

51. Zhu, S., Y. Wu, Z. Yu, J. Liao and Y. Zhang. 2005. Pretreatment by microwave/alkali of rice straw and its enzymatic hydrolysis. *Process Biochemistry* 40: 3082 – 3086.

52. Zhu, S., Y. Wu, Z. Yu, X. Zhang, C. Wang, F. Yu and S. Jin. 2006. Production of ethanol from microwave-assisted alkali pretreated wheat straw. *Process Biochemistry* 41: 869 – 873.

Chapter 4

4. Microwave-Assisted Alkali Pretreatment and Enzymatic Saccharification of Canola straw and Oat hull.

Some portion of this chapter has been published in peer-reviewed journal Bioengineering. A similar version submitted for presentation at the International Conference 2017 Annual General Meeting and Technical Conference of Canadian Society of Biological Engineers:

- Obiora S. Agu, Lope G. Tabil and Tim Dumonceaux. 2017. Microwave-assisted alkali pretreatment, densification and enzymatic saccharification of canola straw and oat hull. *Bioengineering* doi:10.3390/bioengineering4020025
- Obiora S. Agu, Lope G. Tabil, Tim Dumonceaux and V. Meda. 2017. Microwave-assisted alkali pretreatment and enzymatic saccharification of canola straw and oat hull. CSBE/SCGAB 2017 Annual Conference, Paper No. CSBE17-108, Canad Inn Polo Park, Winnipeg, Manitoba, 6 – 10 August.

Contributions of M.Sc. Candidate

The study involves the establishment of a technique that could be used to evaluate the effects of microwave-assisted alkali pretreatment in improving enzymatic saccharification of pretreated lignocellulosic biomass. The biofuel industry can used this technique to evaluate and quantify the glucose present in the analyzed sample. All the experiments, data analysis, and manuscript writing were performed by Obiora Samuel Agu with technical assistance of Dr. Tim Dumonceaux. Dr. Lope Tabil, Dr. Tim Dumonceaux and Dr. Venkatesh Meda provided editorial input to this chapter. Dr. Lope Tabil provided technical direction in experimental design, experimental resources (space and materials) and data analysis. Dr. Tim Dumonceaux provided technical input, experimental resources for chemical composition and saccharification analysis and editorial input to this chapter. In addition, Dr. Lope Tabil established research collaboration with Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatchewan where the glucose analysis was performed.

4.1 Abstract

Microwave-assisted alkali pretreatment was investigated to enhance enzymatic saccharification of canola straw and oat hull for the production of bioethanol. Pretreatment is a necessary step for efficient and effective conversion of lignocellulosic biomass to biofuel. The study compared the effectiveness of microwave-assisted alkali pretreatment, microwave alone and alkali treatment for enzymatic digestibility on canola straw and oat hull. Microwave pretreatments were employed by immersing the biomass in dilute alkali solutions (NaOH and KOH) at various concentrations of 0, 0.75 and 1.5% (w/v) for microwave-assisted times of 6, 12, and 18 min. Alkali treatments were carried out using the same procedure but by soaking and without microwave heating. The highest glucose yields after enzymatic saccharification for both canola straw and oat hull were obtained when these biomass were ground using 1.6 mm hammer mill screen size and subjected to microwave-assisted alkali pretreatment using 1.5% and 0.75% NaOH for 18 min respectively. SEM analysis indicated a more significant modification in the structure of biomass samples subjected to microwave-assisted alkali pretreatment compared to untreated and alkali treated biomass samples. Results indicated that microwave-assisted alkali pretreatment at short residence time enhanced glucose yield.

4.2 Introduction

Bioethanol is a product from sugar-based, corn-based and lignocellulosic materials. Lignocellulosic biomass includes agricultural wastes and crop residues which are widely available and abundant at low cost (Gong et al. 2010; Demirbas et al. 2009; Tong et al. 2012). Liquid biofuel can replace the fossil fuels used in transportation, electricity, heat and plant generation for domestic and industrial purposes and, ethanol current blend has facilitated positive ethanol-petrol mixtures (Ohgren et al. 2007). In Canada, 5% renewable content in gasoline since 2010 and 2% renewable content in diesel fuel and heating oil since 2011, have been implemented using ethanol blends (Liu et al. 2014; Sorda et al. 2012). In U.S., 10% bioethanol in total gasoline consumption by 2020 has been targeted to produce 136 billion liters of biofuel (Liu et. 2014). However, it is clear that the biomass feedstocks for production of bioethanol has shifted from first generation feedstocks (grains and oilseeds) to second (cellulosic biomass from crop residues and dedicated energy crops) and third generation feedstocks (microalgae) in order to promote renewable energy production (Smith 2013; Liu et al. 2014). The second and third

generation feedstocks have been attracting research interest because they are from feedstock that are non-food materials and have no competition with food.

Bioethanol produced from biomass for renewable energy helps to reduce greenhouse gas (GHG) emissions by replacing fossil fuel combustion (Liu et al. 2014) and due to the environmental challenges with fossil fuel usage, research scientists are exploring ways of using cellulosic biomass for bioethanol and biofuel production (Nomanbhay et al. 2013; Diaz et al. 2015) which will reduce the over dependency on fossil fuels usage and reduce global warming, a major contributor to acid rain and climatic changes. Fossil fuels are high in sulfur and nitrogen which form sulfur dioxide and nitrous oxides. These emissions result in acid rain which can damage fresh water sources, forests, soils and adversely affect human health (Demirbas 2004). Therefore, to overcome these problems, there is a need to replace the existing energy source with a more reliable, renewable and environmentally friendly energy source.

Agricultural crop residues are potential biomass that can be used for sustainable production of bioethanol and biofuel (Adapa et al. 2009) and access is virtually unlimited, low cost and readily available (Gong et al. 2010; Demirbas et al. 2009). These agricultural wastes and crop residues are categorized in six groups: crop residues (corn stover, wheat straw, rice straw, barley straw, rice hulls, sweet sorghum bagasse), hardwood (aspen, poplar), softwood (pine, spruce), cellulose wastes (paper waste, recycled paper sludge), herbaceous biomass (alfalfa hay, switchgrass, bermudagrass, thimothy, miscanthus), and municipal solid waste (Sanchez and Cardona 2008). Total biomass production in the Canadian agricultural sector required for energy production was estimated to be 37.3 Mega gram (Mg), and was dominated by crop residues.

In order to utilize the biomass, the structural cell wall of the biomass need to be disrupted and disintegrated, create access for cellulose and hemicellulose to be hydrolyzed into fermentable sugars for bioethanol production. The conversion of lignocellulosic bioethanol process is categorized into four steps: pretreatment, enzymatic saccharification, fermentation and product (ethanol) recovery (Quintero et al. 2011; van Zessen et al. 2003). Over the years, the aim of research investigations on pretreatment technique is to develop pretreatment methods to improve biomass digestibility for subsequent enzymatic saccharification (Ma et al. 2009). Thus, the various pretreatment methods developed include alkali and microwave assisted pretreatment, dilute acid, steam explosion, ammonia fiber explosion (AFEX), lime treatment and organic

73

solvent treatments; these methods assist in the breakdown or altering of lignin or hemicellulose, decrease in cellulose crystallinity and surface area increase to enhance saccharification of cell wall carbohydrates (Quintero et al. 2011; Mosier et al. 2005). Enzymatic hydrolysis process applied is to convert polysaccharides; cellulose and hemicellulose, and oligomers into simple sugars.

Microwave irradiation has gained application in research studies because of its easy operation and high heating efficiency. Microwave heating is an alternative and energy efficient procedure when compared to the conventional heating (Hu and Wen 2008). The electromagnetic field directly interacts with the molecular structure of the heated object (Hu and Wen 2008) and rapidly oscillating electric field in the microwave spins polar molecules especially water thereby disordering the ions with a frequency of $10^6 - 10^{10}$ GHz and causing heat dissipation (Chen et al. 2012). Ethaib et al. (2015) reported that microwave pretreatment combines both thermal and non-thermal effects within the aqueous environment of physical, chemical or biological reactions. Pretreatment of lignocellulosic biomass using microwave heating is done selectively especially at the polar parts and results increase in disruption of recalcitrant structures of the biomass (Tomas-Pejo 2011). According to Alvira et al. (2010), microwave pretreatment technology shows high increased accessibility of surface area, cellulose decrystallization, lignin removal and structure alteration, and low hemicellulose solubilization and toxic compounds generation on the structure of lignocellulose biomass compared to other pretreatment methods. First discovery of microwave pretreatment on biomass was reported by Ooshima et al. (1984) and Azuma et al. (1984) on rice straw and bagasse. The result showed 1.6 times increase in enzymatic accessibility and 3.2 times for the pretreated samples compared to untreated samples (Hu and Wen 2008; Xu 2015; Chen et al. 2012). Reports from previous study indicated that microwave heating though at different operating parameters with respect to power level, residence time and temperature could change the ultra-structure of cellulose, degrade lignin and hemicellulose in lignocellulosic biomass, and increase the enzymatic susceptibility of cellulosic biomass (Binod et al. 2012; Nomanbhay et al. 2013; Choudhary et al. 2012). Also, microwave pretreatment in the presence of water could improve the enzymatic hydrolysis of lignocellulosic biomass (Binod et al. 2012) and with alkali or acid, the results vary significantly with different feedstocks used (Choudhary et al. 2012). The combination of microwave and chemical pretreatment on different biomass as reported by several research studies indicated good sugar

recovery, and various chemicals used in this process are dilute ammonia, iron-chloride and the common ones, alkaline and acid (Xu 2015). The chemicals assist microwave pretreatment method is to remove lignin (alkali solution) and hemicellulose (acid solution) for cellulose accessibility (Ethaib et al. 2015). Therefore, in this study, alkali (sodium and potassium hydroxides) solution was used. The sugar yield of alkaline pretreatment is dependent on the feedstock used. However, biomass used for pretreatment process tends to react with some of the alkali and it leads to solubilization, swelling, increase in internal surface of cellulose, decrease in degree of polymerization and crystallinity, and disruption of lignin structure (Taherzadeh and Karimi 2008; Quintero et al. 2011). Microwave-assisted NaOH pretreatment is commonly used as pretreatment chemical for lignocelluloses. This is due to its ability to delignify biomass and in large scale process, it may not be cost effective (Sindhu et al. 2015). Microwave-assisted KOH pretreatment is not commonly used but was used with switch grass at very low concentration and was found effective resulting in high sugars during hydrolysis (Sharma et al. 2013).

However, it was observed from previous studies that there is knowledge gap in the application of microwave-assisted alkali pretreatment and enzymatic saccharification on canola straw and oat hull. The objective of this study was to investigate the effect of microwave-assisted alkali pretreatment of canola straw and oat hull on their enzymatic digestibility as represented by glucose yield. Canola straw and oat hull which were subjected to microwave-assisted alkali pretreatment, microwave (distilled water) pretreatment and alkali treatment (no heating) to assess their effectiveness in terms of glucose yield during enzymatic saccharification.

4.3 Materials and Methods

4.3.1 Sample Preparation

The canola was a hybrid variety (LL252) and harvested on September 23, 2015 on NW 02-43-13-W3 100 km NW of Saskatoon in the Black Soil Zone. The canola straw was collected from the same zone and oat hull was sourced from (Richardson Milling Ltd.) Martensville, Saskatchewan (52.29^{o}N, 106°W) and were stored at room temperature. The samples each were ground using a hammer mill (Glen Mills Inc. Clifton, NJ) with a screen size of 1.6 and 3.2 mm. The moisture content, bulk and particle densities, and particle size analysis were determined and results were reported in Chapter 3.

4.3.2 Microwave-Assisted Alkali Pretreatment

Microwave (MW) treatments were carried out using a domestic microwave oven (Model NNC980W, Panasonic Canada Ltd, Mississauga, ON, Canada) with an operating frequency of 2450 MHZ and variable power from 220 to 1100 W. The microwave heating temperature data recording and acquisition in the experiment was done using Qualitrol Corporation software (Quebec City, QC, Canada) and the Nomad fiber optic thermometer (Model NMD228A, Quebec City, QC, Canada). The data logging was one data point for every 5 s. Twenty grams of ground biomass sample (canola straw or oat hull) was immersed in 180 g of various alkaline solutions of 0, 0.75 and 1.5% (w/v) NaOH and 0, 0.75 and 1.5% (w/v) KOH at a ratio of 1:9. The mixture was placed in a 600 ml beaker and biomass mixture allowed to absorb the alkaline solution for a period of 30 to 45 min. The mixture was placed at the center of rotating ceramic plate inside the microwave oven for treatment at a fixed power of 713 W (Kashaninejad and Tabil 2011). The temperature probe was inserted through a hole closed with a cork on top of the microwave oven and inserted half way into the beaker containing the sample. The mixture was exposed to three levels of residence time 6, 12 and 18 min, and temperature reading recorded accordingly. The process was done in five replicates for each sample. After the treatments, the moisture content of each sample was determined. The samples were dried and maintained at appropriate moisture level of 12% (w.b.) using forced-air convection dryer set at 42°C (Iroba et al. 2013) and stored in Ziploc bag.

The chemical compositions of the microwave-assisted alkali pretreated, microwave alone pretreated and alkali treated were analyzed using the Ultra Performance Liquid Chromatography (UPLC – MS system Acquity 2004 – 2010, Water Corp., Milford, MA, USA) based on National Renewable Energy Laboratory standard (NREL – Sluiter et al. 2007). The analysis was done in a two-stage acid hydrolysis, with 72% H_2SO_4 and 4% H_2SO_4, in order to fractionate the biomass into forms that are quantifiable (Iroba and Tabil 2013). Prior to this analysis, the biomass samples at 11 – 12% (wb) were dried at 105°C in an air-oven (Thermo Scientific model No. PR305225M; Marietta, OR, USA.) for 24 h. The extractive removal was done by adding the sample to a filter paper pouch, refluxed with acetone using a Soxhlet apparatus for 24 h. The acetone washed sample was left at room temperature for about 3 – 4 h in order to allow acetone to evaporate and then followed by oven drying at 105°C for 24 h. And 300 mg of each oven dried extractive free sample was added into a 100 ml glass pressure tube and 3 ml of 72% H_2SO_4

was added. The mixture was macerated with glass rod every 10 min for 2 h. The acid mixture was diluted to 4% by adding 84 ml of distilled water and autoclaved for 2 h. The sample was allowed to cool at room temperature and separated using side-arm flask vacuum filtration device into hydrolysate (soluble material) and retentate (insoluble material). The retentate inside the crucible of each sample was dried at 105°C for 24 h and subsequently cooled in a desiccator before the weight was determined and recorded. Acid insoluble lignin content was evaluated based on the NREL protocol as presented in equation (4.1).

$$\text{Insoluble lignin content} = \frac{(dried\ retentate)}{(dried\ sample)} \text{ x } 100\% \qquad (4.1)$$

The acid soluble lignin was measured using UV – Vis spectroscopy (BIOMATE 3S, Thermo Fisher Scientific, Madison, WI, USA) at an absorbance of 240 nm. 30 ml of the hydrolysate was neutralized by adding 1 g of $CaCO_3$ and mixed. The mixture was allowed to settle for 5 min and 10 ml of the liquid fraction was carefully poured into a 15 ml centrifuge and subsequently centrifuged at 150 rev/min for 2 min, 1 ml of cleared supernatant was collected and stored at -20°C before monosaccharide quantifications using the Water Acquity UPLC – MS system (Acquity 2004 – 2010, Water Corp., Milford, MA, USA). Sample preparation for monosaccharide quantification was: 100 μl of stored neutralized hydrolysate with 800 μl of 75% acetonitrile/25% methanol and 100 μl of fucose solution (~1 mg/ml) and filtered through 0.2 μm filter into a 2 μl UPLC vial. The LC conditions for the monosaccharide quantification were: Acquity UPLC BEH Amide column (1.7 μm pore size, 2.1 X 50 mm); 0.25 ml min^{-1} flowrate; mobile phase A: 95% acetonitrile/5% isopropanol; mobile B: 80% acetonitrile/0.1% NH_4OH; gradient of 100% A to 100% B over 10 min, then gradient of 100% B to 100% A over 4 min (14 min total run time per sample). The UPLC - MS conditions for the same monosaccharide quantification were: 2.8 kV; 25 V (cone); 50 1 h^{-1} (cone); gas flow 600 1 h^{-1}; desolvation temperature 350 ^{o}C; source temperature 120°C; and dwell time 0.08 s.

At the concentrated acid stage, the polymeric carbohydrates (cellulose and hemicelluloses) were hydrolyzed into monomeric forms (xylose, arabinose, mannose, glucose and galactose), soluble in the hydrolysis liquid and were measured by UPLC. The standards of the monomeric sugars were prepared and evaluated using the UPLC. The spectra of mannose, glucose, and galactose displayed at a molecular weight 179.2 g/mol while xylose and arabinose

77

displayed at a 149.1 g/mol. The correlated monosaccharide peak extracted from the integrated peak area was used to pre-determine regression equations from dilution series of the monosaccharide standards using Microsoft Excel.

The monomeric sugars regression analysis was determined using regression approach, the sugar content evaluated using equation (4.2).

$$\text{Sugar content} = \frac{sugar\ concentration\ \times 87\ ml\ \times 10\ \times H}{dried\ sample\ mass} \text{ x } 100\% \qquad (4.2)$$

where, H could be 0.88 or 0.90 depending on the number of carbons present in the sugars which accounts for the water molecule added during the hydrolysis. 5-carbon sugars (pentoses: xylose and arabinose) and 6-carbon sugars (hexoses: mannose, galactose and glucose) values were multiplied by anhydro correction factor of 0.88 and 0.90, and replicated three times for each sample.

After the chemical compositions analysis followed enzymatic saccharification experiment (NREL – Selig et al. 2008) and subsequently subjected to glucose analysis using dinitrosalicylic (DNS) acid method (Miller et al. 1959; Wood et al. 1988; Xiao et al. 2004).

4.3.3 Alkali Treatment

Twenty grams of ground biomass samples (canola straw or oat hull) was immersed in 180 g of various alkali solutions of 0, 0.75 and 1.5% (w/v) NaOH and 0, 0.75 and 1.5% (w/v) KOH. The mixture was placed in a 600 ml beaker cover with aluminum foil and kept on a platform using the same time with microwave pretreated samples. After the treatment, the moisture content was determined (ASABE standard S358.2 (2006)). Samples were dried and conditioned as indicated in microwave-assisted alkali pretreatment. Figure 4.1 shows the experimental set-up. The process was done in three replicates for each sample. The chemical composition was also determined as indicated in microwave-assisted alkali pretreatment.
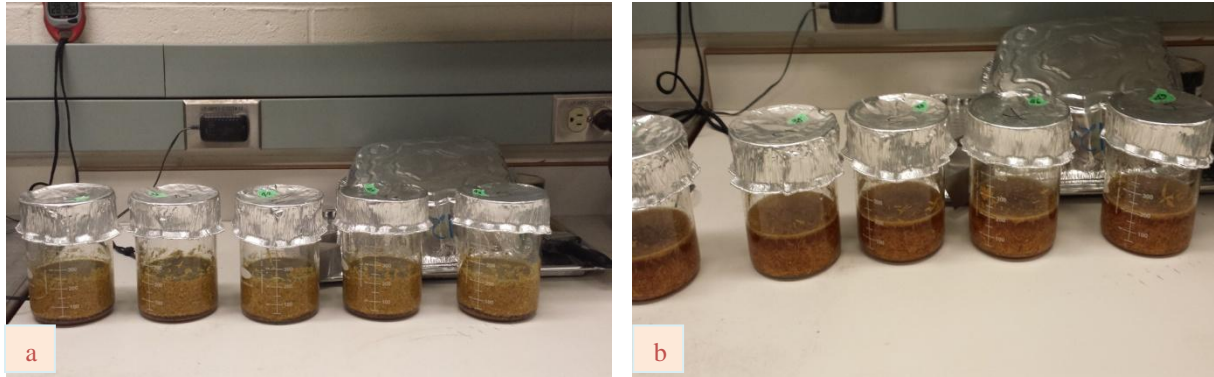
Figure 4.1. Canola straw (a) and oat hull (b) soaked in alkali solutions

4.3.4 Enzymatic Saccharification

The enzymatic saccharification analysis was performed using the dinitrosalicylic acid (DNS) method for estimating reducing sugar (Wood et al. 1988). The enzymes used were cellulase (C2730-50 ml, cellulase from *Trichoderma reesei* ATTC 26921, Sigma-Aldrich Co., St. Louis, MO, USA) and β-glucosidase (C6105-50 ml, Novozyme 188, Sigma-Aldrich Co., St. Louis, MO, USA). The addition of β-glucosidase was necessary to mitigate cellobiose inhibition of cellulase and cellobiose is a disaccharide consisting of two glucose molecules linked by a β-1, 4-glycoside bond (Ryu and Mandels 1980; Nomanbhay et al. 2013). To determine the cellulose activity in suitably diluted sample, the filter paper assay was done to ascertain the filter paper unit (FPU) of the cellulase enzyme (equ. 4.3) to be used in evaluating the average of one μmole of glucose equivalents released per min in the assay reaction (Xiao et al. 2004).

$$\text{FPU/ml} = \left(\frac{A_{540 Sample}}{A_{540}/mg\ standard} \right) (5.55\ \mu mole/mg) \times \left(\frac{1}{60\ min} \right) \left(\frac{1}{\text{Xml}} \right) \qquad (4.3).$$

where FPU/ml is the determined cellulose activity; A_{540} sample is the absorbance obtained from the DNS assay for each cellulase assay; A_{540}/mg standard is the absorbance for 1 mg of glucose measured from the glucose standard curve; 5.55 μmole/mg is the number of μmoles of glucose in 1 mg; 60 min assay incubation time, and X ml (0.02 ml) volume of suitably diluted cellulase that was assayed.

The enzyme mixture for the saccharification assay was prepared in a 10 ml clear scintillation vial tube such that 0.25 ml enzyme contains 85.54 FPU/ml cellulase (0.93 ml), 300 CBU/ml Novozyme 188 (0.53 ml) and 0.54 ml sodium acetate buffer (50 mM, pH 4.8) for the digestion.

One gram biomass sample was weighed and transferred into 50 ml flasks containing 19.75 ml sodium acetate (NaAc) buffer (50 mM, pH 4.8). The pH reading for enzymatic cellulose saccharification of lignocellulosic substrates was in range with the substrate suspension pH 5.2 – 6.2 (Lan et al. 2012). To each flask, 100 µl of a 2% sodium azide solution were added and this was used to prevent microbial growth during digestion. The 0.25 ml enzyme mixture was added last since the reaction is initiated by the introduction of the enzyme. The reaction blank for the substrate (without enzyme) was prepared with the same amount of biomass and buffer. The flasks were tightly closed, placed in a scintillation vial rack, and placed in a shaking incubator (Classic series C24, serial No. 790860283, New Brunswick Scientific Co. Inc. Edison, NJ, USA). The temperature was set to 50°C and incubation was done for 72 h with shaking/rotation at 250 rev/min, which is sufficient to keep solids in constant suspension within the incubation period for the release of soluble sugars from the samples. At the end of incubation, the sample was allowed to cool at room temperature. A 20 µl aliquot was collected and prepared for micro-plate DNS glucose analysis. Three replicates of each sample were performed.

4.3.5 Micro-Plate DNS Glucose Analysis

The glucose (total reducing sugar) was analyzed using a micro-plate modified DNS assay as described according to Wood et al. (1988) and Xiao et al. (2004). In order to determine the standard curve of the amount of glucose in each well, a 60-µl format assay was used. The reason is because it is highly reproducible, accurate and easily assay a large number of samples compared to standard and 96 µl filter paper assay protocols (Xiao et al. 2004). Using a 60 µl format assay, 20 µl aliquot of the sample was added into PCR micro-plate (Thermowell Fisher, Ottawa, ON, Canada) wells containing 40 µl of 50 nM NaAc buffer (pH 4.8) and 120 µl DNS solution was added to each well. The plate was covered with a thermowell sealer and incubated at 95°C for 5 min. After incubation, 36 µl aliquot of each digested sample was transferred to a 96-well flat-bottomed micro-plate (Corning Inc., Corning, NY, USA) containing 160 µl of water

and A_{540} nm was measured (Spectra Max-Plus, Sunnyvale, CA, USA). The glucose mean A_{540} nm was used to calculate the expected A_{540} for 1 mg glucose digested using equation 4.4.

G (mg/g) in one gram sample = A_{540}/mg standard x A_{540} sample x C x D   (4.4).

where G is the expected amount of glucose digested in one gram sample; A_{540}/mg standard is the extrapolated value from the glucose standard curve for 1 mg of glucose and A_{540} sample is the average mean value from the absorbance measurement. C is equal to 0.9 mg/ml, the correction for hydration (to correct for the water molecule added upon hydrolysis of the cellulose polymer) and D is equal to 10 ml, the total volume of assay. The glucose digestion percentage was calculated using equation 4.5.

$$\text{Glucose digestion \%} = \frac{cellulose\ digested\ (mg)}{cellulose\ added\ (mg)}\ x\ 100 \qquad (4.5)$$

4.3.6 Scanning Electron Microscopy of Biomass Sample

The structural changes of microwave-assisted pretreated, microwave pretreated, alkali treated and untreated ground canola straw and oat hull were observed by JEOL, JSM-6010LV scanning electron microscope (SEM) (JEOL USA, Inc., Peabody, MA, USA) at an accelerating voltage of 5 kV. The samples were coated with a thin conducting layer of ~ 10 – 100 nm gold sputter. The coating was achieved through sputtering by plasma under vacuum (Model S150B, Sputter Coater, Edwards, NY, USA). This is to improve sample electronic conductivity during imaging (Oguocha 2015). The fine coated specimens were fixed on the stub with adhesive and observed.

4.4 Results and Discussion

4.4.1 Lignocellulosic Biomass Characterization

Physical properties results were reported in Chapter 3 and Tables 4.1 and 4.2 show the lignocellulosic composition of microwave-assisted alkali pretreated, microwave alone (distilled water) and alkali treated canola straw and oat hull samples. The composition of canola straw was determined to be 42.39% cellulose, 16.41% hemicellulose and 14.15% lignin (Adapa et al.

2009), whereas oat hull had a composition of 34.61% cellulose, 29.80% hemicellulose and 10.01% lignin (Gerwal et al. 2015). Reports from previous studies stated that alkali treatments dissolves lignin and hemicellulose, and microwave heating enhances breakdown of these components in alkali solutions (Kashaninejad and Tabil 2011; Kumar et al. 2009). The cellulose content increased with increasing alkali concentration and microwave heating time whereas the lignin content decreased with increase in microwave heating time and alkali concentration. In alkali treatment, the cellulose content increased with decreasing alkali concentration and lower soaking time whereas the hemicellulose and lignin contents decreased with higher soaking time and alkali concentration. This implies that there is a breakdown of the biomass matrix in the lignin and creates accessibility and digestibility of cellulose and hemicellulose (Kumar et al. 2009; Taherzadeh and Karimi 2008). The lignin content of pretreated canola straw and oat hull samples was lower than microwave alone pretreated and alkali treated samples. The lignin decrease indicates solubilization in the alkaline aqueous solution and increase in cellulose was as a result of solubilization from other components in the alkali solution. Also, increase in cellulose content by microwave heating was facilitated by dissolution of components in alkaline solutions (Zhu et al. 2005; Zhu et 2006). The microwave-assisted alkali pretreatment removed more hemicellulose and lignin in canola straw than oat hull samples and similar results were indicated in alkali treatment. In addition, microwave-assisted alkali pretreatment in both feedstocks resulted in higher solubilization of cellulose and, decrease in hemicellulose and lignin. The stronger alkali pretreatment in combination with long microwave heating and soaking time caused more solubilization of cellulose, hemicellulose and lignin. Zhu et al. (2006a and 2005) reported a similar result with wheat straw and rice straw. For the both feedstocks in this study, canola straw samples showed higher solubilization with the alkali solution than the oat hull in microwave-assisted alkali pretreatment and alkali treatment. This shows that alkali used in the microwave pretreatments and alkali treatments caused swelling and lignin structure disruption in the biomass that resulted to solubility of lignin in the feedstocks (Taherzadeh and Karimi 2008; Tomas-Pejo et al. 2011). Kashaninejad and Tabil (2011) reported that the main aim of using alkali solution during microwave pretreatment method is to disintegrate the ester bonds between lignin and carbohydrate in the biomass and this statement is supported with the data presented herein.

Table 4.1. Chemical composition (% dry basis) of microwave-assisted alkali pretreated canola straw and oat hull.

Sample	Screen size (mm)	Alkali	Concentration (%)	MW heating time (min)	Cellulose[a] (%)	Hemicellulose[a] (%)	Lignin[a] (%)
Canola straw	1.6		0	18	63.1 ± 32.0	5.5 ± 6.3	5.0 ± 0.8
		NaOH	1.5	18	59.1 ± 0.5	9.4 ± 8.3	4.3 ± 1.2
			1.5	6	37.8 ± 3.1	7.2 ± 6.5	4.7 ± 0.6
		KOH	0.75	12	53.6 ± 9.2	10.6 ± 9.2	5.8 ± 0.3
			1.5	6	56.9 ± 17.0	7.7 ± 9.0	4.6 ± 0.5
	3.2		0	18	60.0 ± 21.8	6.2 ± 5.5	5.6 ± 1.0
		NaOH	0.75	12	54.2 ± 2.3	6.7 ± 5.8	5.1 ± 0.6
			0.75	6	38.2 ± 2.7	8.7 ± 7.5	5.3 ± 0.3
		KOH	0.75	12	30.8 ± 2.9	13.8 ± 13.0	5.0 ± 1.6
			1.5	6	63.4 ± 35.0	10.3 ± 9.2	4.4 ± 0.5
Oat hull	1.6		0	12	36.7 ± 17.0	10.5 ± 9.1	7.5 ± 1.2
		NaOH	0.75	18	42.8 ± 11.3	15.6 ± 13.8	6.3 ± 1.0
			1.5	18	37.1 ± 8.5	14.3 ± 12 .6	4.2 ± 1.2
		KOH	1.5	18	56.4 ± 17.9	16.0 ± 13.8	4.8 ± 0.9
			1.5	6	41.8 ± 14.0	12.9 ± 11.5	5.7 ± 1.6
	3.2		0	6	51.2 ± 19.5	11.0 ± 9.6	9.2 ± 0.4
		NaOH	0.75	6	22.7 ± 11.0	12.9 ± 14.4	6.8 ± 2.2
			1.5	18	48.7 ± 8.3	14.4 ± 13.3	5.1 ± 0.8
		KOH	0.75	12	47.9 ± 18.2	16.0 ± 16.0	5.4 ± 0.6
			1.5	18	62.6 ± 2.0	10.4 ± 18.0	6.4 ± 1.3

MW – microwave.
[a] Mean ± standard deviation of three replicates.

Table 4.2. Chemical composition (% dry basis) analysis of alkali treated canola straw and oat hull.

Sample	Screen size (mm)	Alkali	Concentration (%)	Soaking time (min)	Cellulose[a] (%)	Hemicellulose[a] (%)	Lignin[a] (%)
Canola straw	1.6	NaOH	0.75	6	79.9 ± 1.9	8.5 ± 7.5	7.9 ± 1.4
			0.75	18	69.7 ± 5.1	6.0 ± 5.2	6.6 ± 4.1
		KOH	1.5	12	69.2 ± 1.0	9.8 ± 8.6	8.8 ± 1.2
			1.5	18	61.3 ± 16.7	8.2 ± 9.0	8.3 ± 1.4
	3.2	NaOH	1.5	6	54.1 ± 6.7	10.4 ± 11.2	9.2 ± 1.6
			1.5	18	82.2 ± 3.9	7.3 ± 7.0	6.9 ± 3.0
		KOH	0.75	6	68.1 ± 8.4	9.2 ± 9.1	9.1 ± 0.9
			1.5	6	46.6 ± 1.5	9.7 ± 8.4	8.2 ± 1.2
Oat hull	1.6	NaOH	0.75	18	67.9 ± 23.1	14.4 ± 14.6	11.5 ± 0.8
			1.5	12	62.4 ± 0.1	21.4 ± 23.6	11.2 ± 2.7
		KOH	0.75	6	64.7 ± 1.4	12.0 ± 11.0	10.9 ± 2.1
			0.75	12	37.0 ± 18.8	10.3 ± 10.6	13.4 ± 1.8
	3.2		0	6	41.5 ± 2.1	9.5 ± 8.4	11.7 ± 1.3
		NaOH	1.5	6	66.9 ± 8.3	20.1 ± 17.4	9.8 ± 3.4
			1.5	18	57.0 ± 3.5	14.1 ± 13.2	11.8 ± 0.6
		KOH	0.75	12	57.2 ± 17.2	24.8 ± 23.7	13.7 ± 2.3

[a] Mean ± standard deviation of three replicates.

4.4.2 Glucose Yield

Microwave-assisted alkali pretreated, microwave alone and alkali pretreated canola straw and oat hull samples were used as substrates for enzymatic saccharification. These substrates were subjected to enzymatic saccharification in order to convert cellulose to glucose. Saccharification of cellulosic biomass prior to fermentation to ethanol is very important step because the yeast (*S.cerevisiae*) used is a cellulolytic microbe (Gupta et al. 2012). Tables 4.3 and 4.4 show the glucose yields in one gram of the dry biomass samples (canola straw and oat hull). The data validates the effectiveness of the pretreatment method by reflecting the accessibility and digestibility of cellulose (glucose) in the microwave-assisted alkali pretreated samples compared to microwave alone pretreated and alkali treated samples. However, the DNS method only quantifies glucose. But the substrate contains other sugar contents such as mannose, galactose and hexoses. The highest glucose (sugar) yield (110.05 mg/g) for one gram canola straw sample was obtained from microwave-assisted alkali pretreatment with 1.5% NaOH for 18

min; the sample was ground using 1.6 mm hammer mill screen size. For 1.5 and 0.75% NaOH and KOH, the yield significantly increased with longer microwave heating time for canola straw ground using 1.6 mm screen size whereas those ground in 3.2 mm screen size, the glucose yield significantly increased with lower microwave heating time. In alkali treatment, the glucose yield significantly increased with longer soaking time in treatments 0.75 and 1.5% NaOH and KOH for canola straw ground using 1.6 mm screen size. The highest glucose yield (99.10 mg/g) for one gram oat hull sample was obtained from microwave-assisted alkali pretreatment with 0.75% NaOH for 18 min; the sample was ground using 1.6 mm hammer milled screen size. Sugar yields increased as the microwave heating was extended from 6 to 18 min with treatment 0.75% NaOH and decreased in treatment combinations 1.5% NaOH/1.6 and 3.2 mm hammer mill screen sizes. Moreover, treatment of 1.5% KOH using 1.6 mm hammer milled oat hull resulted in significantly increased sugar yields with lower microwave heating time, whereas oat hull ground with 3.2 mm screen size treated with 1.5% NaOH alkali solution resulted in high glucose yield with lower soaking time.

In addition, microwave alone (distilled water) pretreated samples showed lower glucose yield compared to microwave-assisted alkali pretreated and alkali pretreated samples. The glucose yield in the microwave alone pretreatment increased as the microwave heating time was extended from 6 to 18 min in both samples. Consequently, the results revealed that in 0.75 and 1.5% NaOH and KOH treatments combinations peak glucose yields were obtained at 18 min of microwave heating time for samples ground with 1.6 mm hammer mill screen size. Also, at initial microwave heating time of 6 min, glucose yield significantly increased with increasing alkali concentration in both treatments.

Table 4.3. Glucose yield of microwave-assisted alkali pretreated canola straw and oat hull.

Sample	Screen size (mm)	Alkali	Concentration (%)	MW heating time (min)	Cellulose in substrate[a] (%)	Average A540 mean value[a] (mg glc.)	Average glucose[a] (mg/g)	Average glucose digestion percentage[a] (%)
Canola straw	1.6		0	18	63.1 ± 32.0	0.574 ± 0.08	42.25 ± 5.99	6.70 ± 0.95
		NaOH	1.5	6	37.8 ± 3.1	0.128 ± 0.04	9.45 ± 2.93	2.50 ± 0.78
			1.5	18	59.1 ± 0.5	1.494 ± 0.12	110.05 ± 9.10	18.62 ± 1.54
		KOH	0.75	12	53.6 ± 9.2	0.338 ± 0.23	24.92 ± 16. 61	4.65 ± 3.10
			1.5	6	56.9 ± 17.0	0.725 ± 0.08	53.42 ± 6.07	9.39 ± 1.07
	3.2		0	18	60.0 ± 21.8	0.662 ± 0.16	48.75 ± 11.99	8.13 ± 2.00
		NaOH	0.75	6	38.2 ± 8.7	0.757 ± 0.14	55.78 ± 10.25	14.60 ± 2.68
			0.75	12	54.2 ± 2.3	0.482 ± 0.30	35.47 ± 22.37	6.54 ± 4.13
		KOH	0.75	12	30.8 ± 2.9	0.434 ± 0.08	31.96 ± 6.10	10.38 ± 1.98
			1.5	6	63.4 ± 35.0	1.314 ± 0.21	96.77 ± 15.31	15.26 ± 2.41
Oat hull	1.6		0	12	36.7 ± 17.0	0.086 ± 0.02	6.33 ± 1.36	1.73 ± 0.37
		NaOH	0.75	18	42.8 ± 11.8	1.346 ± 0.07	99.10 ± 4.79	23.16 ± 1.12
			1.5	18	37.1 ± 8.5	0.031 ± 0.01	2.26 ± 0.70	0.61 ± 0.19
		KOH	1.5	6	41.8 ± 14.0	1.324 ± 0.15	97.53 ± 11.32	23.33 ± 2.71
			1.5	18	56.4 ± 17.9	1.149 ± 0.38	84.64 ± 27.27	15.01 ± 4.96
	3.2		0	6	51.2 ± 19.5	0.073 ± 0.01	5.38 ± 0.51	1.05 ± 0.10
		NaOH	0.75	6	22.7 ± 11.0	0.981 ± 0.11	72.22 ± 7.98	31.82 ± 3.52
			1.5	18	48.7 ± 8.3	0.032 ± 0.01	2.38 ± 0.47	0.49 ± 0.10
		KOH	0.75	12	47.9 ± 18.2	0.452 ± 0.03	33.26 ± 2.51	6.94 ± 0.52
			1.5	18	62.6 ± 2.0	1.152 ± 0.29	84.87 ± 21.25	13.56 ± 3.39

[a] Mean ± standard deviation of three replicates.
MW – microwave.

Furthermore, it was observed that the effect of alkali concentration on the glucose yield varied with microwave heating and soaking time in canola straw and oat hull samples. Microwave pretreatment and alkali treatment using NaOH solution at different concentrations resulted in higher glucose yields compared to KOH in both feedstocks. This implies that NaOH solution with microwave pretreatment was effective to delignify biomass (Sindhu et al. 2015). Also, the data obtained from this investigation revealed that high glucose yields were observed in samples ground using 1.6 mm hammer mill screen size for both feedstocks.

Table 4.4. Glucose yield of alkali treated canola straw and oat hull.

Sample	Screen size (mm)	Alkali	Concentration (%)	Soaking time (min)	Cellulose in substrate[a] (%)	Average A540 mean value[a] (mg glc.)	Average glucose[a] (mg/g)	Average glucose digestion percentage[a] (%)
Canola straw	1.6	NaOH	0.75	18	69.7 ± 5.1	1.1373 ± 0.05	83.76 ± 3.47	12.37 ± 0.51
		KOH	1.5	18	61.3 ± 16.7	1.1960 ± 0.21	88.08 ± 15.34	14.37 ± 2.50
	3.2	KOH	0.75	6	68.1 ± 8.4	0.7173 ± 0.07	52.83 ± 52.83	7.76 ± 0.74
		NaOH	1.5	18	82.2 ± 3.9	0.3547 ± 0.03	26.12 ± 2.25	3.18 ± 0.27
Oat hull	1.6	NaOH	0.75	18	67.9 ± 23.1	0.2993 ± 0.03	22.05 ± 1.94	3.25 ± 0.29
		KOH	0.75	12	37.0 ± 18.8	0.1617 ± 0.03	11.91 ± 1.93	3.22 ± 0.52
	3.2	KOH	0.75	12	57.2 ± 17.2	0.1570 ± 0.02	11.56 ± 1.21	2.02 ± 0.21
		NaOH	1.5	6	66.9 ± 8.3	1.0893 ± 0.20	80.23 ± 14.89	11.99 ± 2.23

[a] Mean ± standard deviation of three replicates.

4.4.3 Morphological and Structural Changes of Biomass

The structural changes that were induced by microwave-assisted alkali pretreatments were investigated by SEM. The examined images of pretreated canola straw and oat hull were compared with microwave alone pretreated and untreated samples at magnification of 250 and 500x. Figures 4.2 and 4.3 (a) – (d) show the observed changes in untreated, microwave alone pretreated, alkali treated and microwave-assisted alkali pretreated canola straw and oat hull surfaces. The SEM in Figures 4.2 and 4.3 (a) showed the undamaged surface of untreated canola straw and oat hull particles, which were smooth, contiguous and intact. In Figures 4.2 and 4.3 (b), the canola straw and oat hull microwaved with distilled water showed slight disorder and disruption on the surfaces compared to the untreated samples. Some opened cell walls were evident and can be recognized. Figure 4.2 and 4.3 (c) showed that the alkali treatment induced physical changes on the surfaces of the biomass. Soaking of canola straw and oat hull in alkali solution caused breakage of cell walls and slight significant erosion of micro-fibrils especially on oat hull samples. Figures 4.2 and 4.3 (d) showed that the microwave-assisted alkali pretreated canola straw and oat hull particles have detached fibers, collapsed cell walls and with porous formation on the individual cell wall transverse plane surfaces. Similar results were reported by Anna and de Souza (2012) and Diaz et al. (Diaz et al. 2015).

Furthermore, the SEM images showed evidence of breakdown of lignocellulosic matrix which is advantageous in releasing the binding agent (lignin) and activating the intermolecular bonds to improve the quality of compressed pellets (Iroba et al. 2014). Also, the images reveal that alkali solution used in the pretreatments caused swelling and disruption of lignin structure in the biomass resulting in enzymatic accessibility and digestibility of cellulose and hemicellulose (Kumar et al. 2009; Taherzadeh and Karimi 2008).

Detached fibers, collapsed and porous formation of cell walls
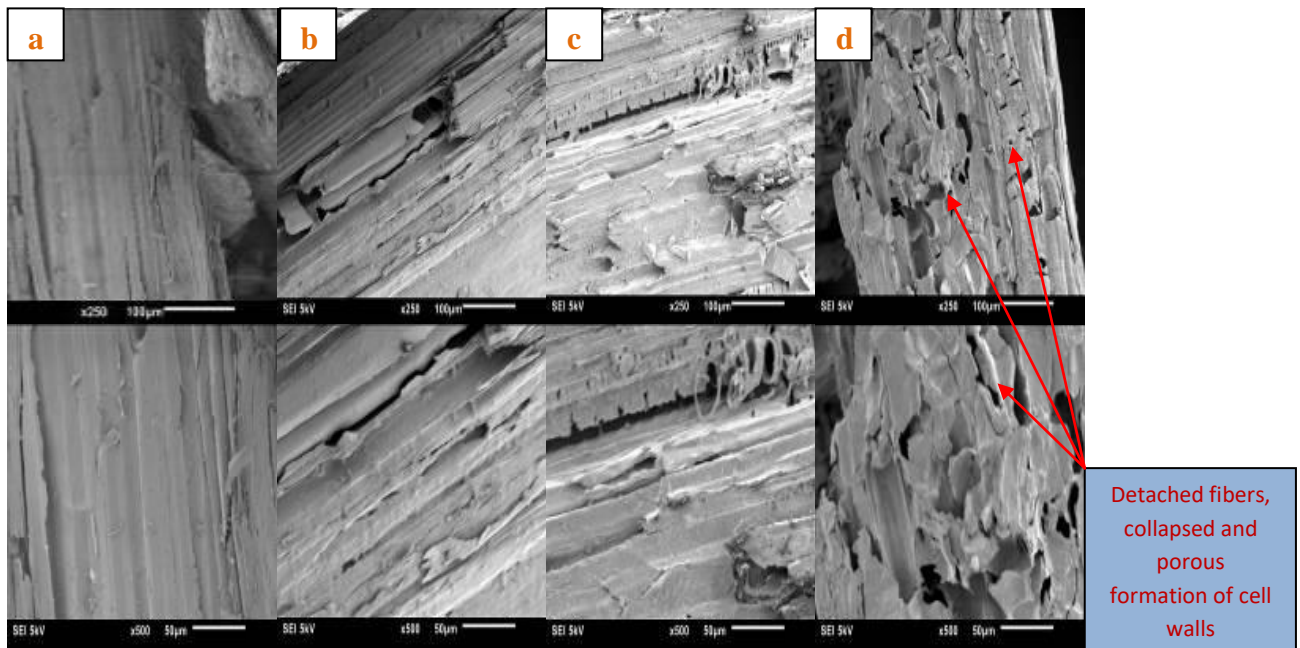
Figure 4.2. SEM images of canola straw at magnifications 250 and 500x. a: untreated sample; b: microwave pretreated with distilled water; c: alkali pretreated; d: microwave-assisted alkali pretreated.
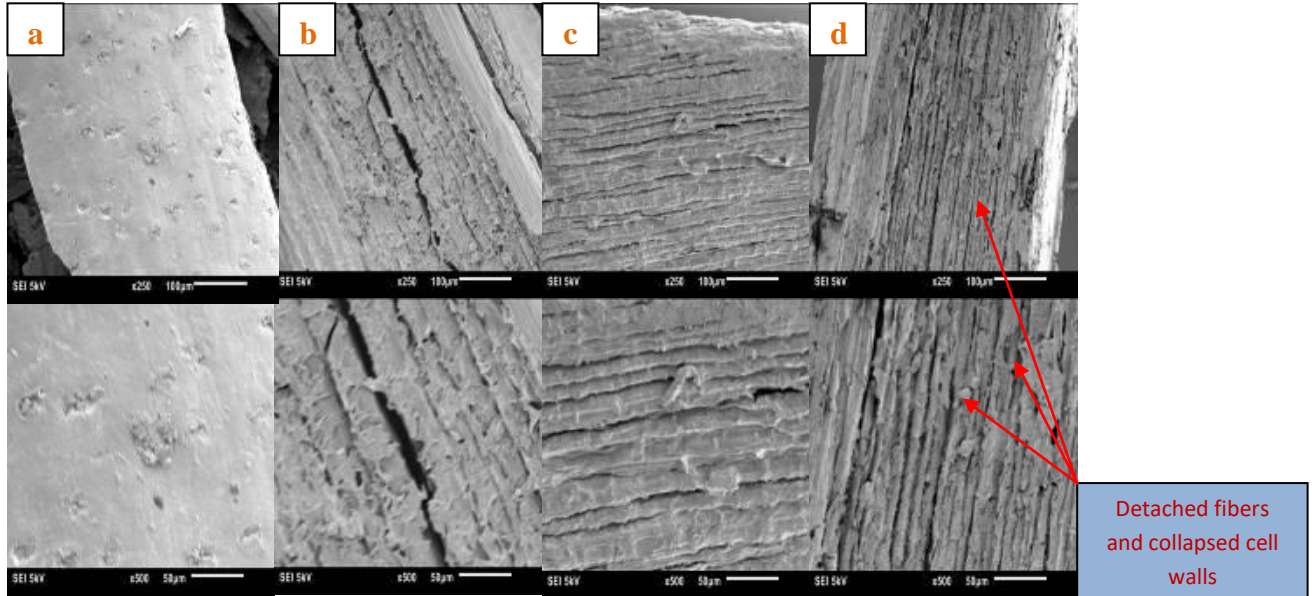
Figure 4.3. SEM images of oat hull at magnifications 250 and 500x. a: untreated sample; b: microwave pretreated with distilled water; c: alkali pretreated; d: microwave-assisted alkali pretreated.

4.5 Conclusions

Enzymatic saccharification of microwave-assisted alkali pretreatment and alkali pretreatment of canola straw and oat hull were investigated, and the following are the conclusions:

1.   Microwave-assisted alkali pretreatment of canola straw and oat hull showed better results in enhancing enzymatic digestibility of substrates compared to alkali pretreatment.

2.   Microwave-assisted alkali pretreatment compared to microwave alone pretreatment and alkali pretreatment was able to disrupt and breakdown the lignocellulosic structure of the samples and created accessible areas for cellulose to cellulase reactivity.

3.   The best enzymatic saccharification result that gave a high glucose yield of 110.0 mg/g dry sample for canola straw was ground in a 1.6 mm screen hammer mill and microwave pretreated with 1.5% NaOH for 18 min. High glucose yield of 99.10 mg/g dry sample for oat hull resulted from those ground in a 1.6 mm screen hammer mill and microwave pretreated with 0.75% NaOH for 18 min.

4. Structural changes of sample particles of microwave-assisted alkali pretreated canola straw and oat hull were observed through SEM images revealing the effectiveness of microwave-assisted alkali pretreatment.

4.6 References

1. Adapa, P., L. Tabil, G. Schoenau, 2009. Compaction characteristics of barley, canola, oat and wheat straw. *Biosystems Engineering* 104: 335 – 344.

2. Alvira, P., E. Tomas-Pejo, M. Ballesteros and M.J. Negro. 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. *Bioresource Technology* 101: 4851 – 4861.

3. ASABE 2006. ASAE S358.2 - Moisture measurement – Forages. In *ASABE Standards*. St. Joseph, MI: American Society of Agricultural and Biological Engineers.

4. Azuma, J.I., F. Tanaka and T. Koshijima. 1984. Enhancement of enzymatic susceptibility of lignocellulosic wastes by microwave irradiation. *Journal of Fermentation Technology* 62(4): 377 – 384.

5. Binod P.; K. Satyanagalakshmi, R. Sindhu, K.U. Janu, R.K. Sukumaran and A. Pandey. 2012. Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse. *Renewable Energy* 37: 109 – 116.

6. Chen, C., D. Boldor, G. Aita and M. Walker. 2012. Ethanol production from sorghum by a microwave-assisted pretreatment. *Bioresource Technology* 110: 190 – 197.

7. Choudhary, R., A.L. Umagiliyage, Y. Liang, T. Siddaramu, J. Haddock and G. Markevicus. 2012. Microwave pretreatment for enzymatic saccharification of sweet sorghum bagasse. *Biomass and Bioenergy* 39: 218 – 226.

8. Demirbas, A., M.F.M. Balat and H. Balat. 2009. Potential contribution of biomass to the sustainable energy development. *Energy Conversion and Management* 50: 1746-1760.

9. Demirbas, A. 2004. 'Bioenergy, global warming, and environmental impacts, energy sources, part a: recovery. *Utilization and Environmental Effects* 26(3): 225 – 236.

10. Diaz, A.B., M.M. Moretti, C. Bezerra-Bussoli, C.daC.C Nunes, A. Blandino, R. da Silva and E. Gomes. 2015. Evaluation of microwave-assisted pretreatment of lignocellulosic biomass immersed in alkaline glycerol for fermentable sugars. *Bioresource Technology* 185: 316 – 323.

11. Ethaib, S., R. Omar, S.M.M. Kamal, D.R.A. Biak. 2015. Microwave-assisted pretreatment of lignocellulosic biomass: A review. *Journal of Engineering Science and Technology* 97 – 109.

12. Grewal, R.K., M. Soleimani and L.G. Tabil. 2015. Investigations on biocomposites from oat hulls and biodegradable polymers. CSBE Paper No. 15-019. Winnipeg, Manitoba: CSBE/SCGAB.

13. Gong, G., D. Liu and Y. Huang. 2010. Microwave-assisted organic acid pretreatment for enzymatic hydrolysis of rice straw. *Biosystems Engineering* 107: 67 – 73.

14. Gupta, P., K. Samant and A. Sahu. 2012. Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. *International Journal of Microbiology* 1 – 5. Doi:10.1155/2012/578925

15. Hu, Z. and Z. Wen. 2008. Enhancing enzymatic digestibility of switchgrass by microwave assisted alkali pretreatment. *Biochemical Engineering Journal* 38: 369 – 378.

16. Iroba, K.L and L.G. Tabil. 2013. Lignocellulosic biomass feedstock characteristics, pretreatment methods and pre-processing of biofuel and bioproduct applications. US and Canada perspectives. In *Biomass Processing, Conversion and Biorefinery*, ed. B. Zhang and Y. Wan, 61-98. New York, NY: Nova Science Inc.

17. Iroba, K.L., L.G. Tabil, S. Sokhansanj and V. Meda. 2014. Producing durable pellets from barley straw subjected to radio frequency-alkaline and steam explosion pretreatments. *International Journal of Agricultural and Biological Eng*ineering 7(3): 68 - 82.

18. Kashaninejad, M. and L. G. Tabil. 2011. Effect of microwave: chemical pretreatment on compression characteristics of biomass grinds. *Biosystems Engineering* 108: 36 – 45.

19. Kumar, P., D. M. Barrett, M. J. Delwiche and P. Stroeve, 2009. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial and Engineering Chemistry Research* 48: 3713 – 3729.

20. Lan, T.Q., H. Lou and J.Y. Zhu. 2013. Enzymatic saccharification of lignocelluloses should be conducted at elevated pH 5.2 – 6.2. *Bioenergy Re*search 6: 476 – 485.

21. Li, X., E. Mupondwa, S. Panigraphi, L.G. Tabil, S. Sokhansanj and M. Stumborg. 2012. A review of agricultural crop residue supply in Canada for Cellulosic ethanol production. *Renewable and Sustainable Energy Reviews* 16: 2954-2965.

22. Liu, T., B. McConkey, T. Huffman, S. Smith, B. MacGregor, D. Yemshanov and S. Kulshreshtha. 2014. Potential and impacts of renewable energy production from agricultural biomass in Canada. *Applied Energy* 130: 222 – 229.

23. Ma, H., W. Liu, X. Chen, Y. Wu and Z. Yu. 2009. Enhanced enzymatic saccharification of rice straw by microwave pretreatment. *Bioresource Technology* 100: 1279 – 1284.

24. Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry* 31: 420 – 428.

25. Mosier, N. S., C. Wyman, B. Dale, R. Elander, Y. Y. Lee, M. Holtzapple and M. R. Ladisch. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology* 96 (6): 673-686.

26. Nomanbhay, S.M., R. Hussain and K. Palanisamy. 2013. Microwave-assisted alkaline pretreatment and microwave assisted enzymatic saccharification of oil palm empty fruit bunch fiber foe enhance fermentable sugar yield. *Journal of Sustainable Bioenergy System* 3: 7 – 17.

27. Oguocha, I. 2015. Materials characterization techniques. Unpublished class material. Saskatoon, Saskatchewan: Department of Mechanical and Material Science Engineering, University of Saskatchewan.

28. Ohgren, K., R. Bura, G. Lesnicki, J. Saddler and G. Zacchi. 2007. A comparison between simultaneous saccharification and fermentation using steam-pretreated corn stover. *Process Biochemistry* 42: 834 – 839.

29. Ooshima, H., K. Aso, Y. Harano and T. Yamamoto. 1984. Microwave treatment of cellulosic materials for their enzymatic hydrolysis. *Biotechnology Letters* 6(5): 289 – 294.

30. Quintero, J.A., L.E. Rincon and C.A. Cardona. 2011. Production of bioethanol from agro industrial residues as feedstocks. In *Biofuels: Alternative Feedstocks and Conversion Processes*, ed. A. Pandey, E. Gnansounou, C.G. Dussap, C. Larroche and S.C. Ricke, 253-260. Oxford, UK: Academic Press.

31. Ryu, D.D. and M. Mandels. 1980. Cellulases-biosynthesis and applications. *Enzyme and Microbial Technology* 2(2): 91 – 102.

32. Sanchez, O.J and C.A. Cardona. 2008. Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresource Technology* 99(13): 5270 – 5295.

33. Selig, M., N. Wesis and Y. Ji. 2008. Enzymatic saccharification of lignocellulosic biomass. Laboratory analytical procedure. NREL/TP-510-42629. Colorado, U.S.A:

National Renewable Energy Laboratory, U.S.A. Department of Energy, Office of Energy Efficiency and Renewable Energy.

34. Sharma, R., V. Palled, R. R. Sharma-Shivappa and J. Osborne. 2013. Potential of potassium hydroxide pretreatment of switch grass for fermentable sugar production. *Applied Biochemistry Biotechnology* 169: 761 – 72.

35. Sindhu R, A. Pandey and P. Binod. 2015. Alkaline treatment. In *Pretreatment of Biomass: Process and Technologies,* ed. A. Pandey, S. Negi, P. Binod and C. Larroche, 51 – 60. Boulevard Langford Lane Kidlington, Oxford UK: Elsevier.

36. Sluiter, A., B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton and D. Crocker. 2007. Determination of structural carbohydrates and lignin in biomass. NREL/TP-510-42618. Golden, CO: Office of Energy Efficiency and Renewable Energy, National Renewable Energy Laboratory.

37. Smith, D. 2013. Brief overview of biofuels. Reports 2 and 3. McGill University, Montreal, QC: Biofuelnet Canada.

38. Sorda, G., M. Banse and C. Kemfert. 2012. An overview of biofuel policies across the world. *Energy Policy* 2(38): 6977 – 88.

39. Taherzadeh, M.J. and K. Karimi. 2008. Pretreatment of lignocellulosic waste to improve ethanol and biogas production: a review. *International Journal of Molecular Sciences* 9: 1621 – 1651.

40. Tomas-Pejo, E., P. Alvria, M. Ballesteros and M.J. Negro. 2011. Pretreatment technologies for lignocellulose to bioethanol conversion. In *Biofuels: Alternative Feedstocks and Conversion Processes,* ed. A. Pandey, E. Gnansounou, C.G. Dussap, C. Larroche and S.C. Ricke, 149-177. Oxford, UK: Academic Press.

41. Tong, Z., P. Pullammanappallil and A.A. Teixeira. 2012. How ethanol is made from cellulosic biomass. AE493. Gainesville, FL: Institute of Food and Agricultural Sciences, University of Florida.

42. Van Zessen, E., M. Weismann, R.R.C. Bakker, H.W. Elbersen, J.H. Reith and H. den. Uil. 2003. Lignocellulosic ethanol, a second opinion. Report 2GAVE-03.11. Wageningen, Netherlands: AFSG Biobased Products, Wageningen University and Research. http://library.wur.nl/WebQuery/wurpubs/339809 (2016/08/18).

43. Wood, T.M., K.M. Bhat, A.W. Willis and T.K. Scott. 1988. Methods for measuring cellulase activities. *Methods in Enzymology* 160: 87 – 112.

44. Xiao, Z., R. Stroms and A. Tsang. 2004. Micro-plate based filter paper to measure total cellulase activity. *Biotechnology and Bioengineering* 832 – 837.

45. Xu, J. 2015. Microwave pretreatment. In *Pretreatment of Biomass: Processes and Technologies*, ed. A. Pandey, 157 – 172. Waltham, MA: Elsevier.

46. Zhu, S., Y. Wu, Z. Yu, J. Liao and Y. Zhang. 2005. Pretreatment by microwave/alkali of rice straw and its enzymatic hydrolysis. *Process Biochemistry* 40: 3082 – 3086.

47. Zhu, S., Y. Wu, Z. Yu, X. Zhang, C. Wang, F. Yu and S. Jin. 2006. Production of ethanol from microwave-assisted alkali pretreated wheat straw. *Process Biochemistry* 41: 869 – 873.

48. Zhu, S., Y. Wu, Z. Yu, Q. Chen, G. Wu, F. Yu, C. Wang and S. Jin. 2006a. Microwave-assisted alkali pretreatment of wheat straw and its enzymatic hydrolysis. *Biosystems Engineering* 93(3): 437 – 442.

Chapter 5

General Discussion

This chapter discusses the results obtained from microwave-assisted alkali pretreatment and alkali treatment, and how the research objectives of this thesis were achieved over the course of the M.Sc. research.

5.1 Overall M.Sc. Project Discussion

This thesis investigated microwave-assisted alkali pretreatment, densification and enzymatic saccharification of canola straw and oat hull. Microwave heating using alkalis (NaOH and KOH) as catalyst at varied heating time and fixed microwave power level (713 W) were the pretreatment approaches used in the research study. The chemical composition (cellulose, hemicellulose and lignin) and pellet quality (density, dimensional stability and tensile strength) of canola straw and oat hull were investigated. Pretreatment and preprocessing methods were used in disintegrating and breaking down of cell walls of the biomass structure. The process of disintegration altered the relative composition of lignin, cellulose and hemicellulose in the material, and consequently, broke down the long-chain hydrogen bond in the cellulose, making hemicellulose amorphous and converting the polysaccharides in to simple sugars through enzymatic saccharification. In addition, the processes also released and caused loss lignin from the lignocellulosic matrix which resulted in better quality pellets. Similar results of lose lignin were reported by Kashaninejad and Tabil (2011) and Iroba et al. (2014). Therefore, pretreatment is required to disrupt and breakdown the structures of lignocellulosic biomass. It assist in loosen up the crystalline structure, increase the biomass porosity, improve the enzyme accessibility and digestibility of cellulose and hemicellulose for glucose production (Keshwani and Cheng 2010; Nomanbhay et al. 2013; Diaz et al. 2015). Consequently, similar results were reported by Ma et al. (2009) and Zhu et al. (2005), where rice straw was used as feedstock.

Particle size reduction facilitates inter-particle bonding and size reduction done at different combinations of grinding and milling, in order to improve enzymatic saccharification of biomass. The bulk and particle densities decreased as the hammer mill screen size of the biomass increased. This may be variation in moisture content and mechanical properties of the different biomass. Also, increasing the hammer mill screen size and alkali concentration increased the

bulk and particle densities of the biomass after pretreatment processes. Kashaninejad and Tabil (2011) reported similar results on the bulk and particle densities, and ash content with increased alkali concentration. The use of the alkali solutions in the pretreatment increased the ash content of the pretreated biomass. The increase was due to high concentration of mineral content of sodium and potassium in the alkali solutions.

The effect of the microwave heating time, sodium and potassium hydroxides were investigated using two biomass screen sizes. The alkalis applied to the lignocellulosic biomass (1:9 ratio) during microwave pretreatment caused swelling of the biomass within the mixture. The addition of alkalis in the microwave pretreatment created more solubilization of cellulose, hemicellulose and lignin of the lignocellulosic biomass as shown in Table 3.5, and it enabled ease of access for enzymatic and microbiological reaction. There was high decrease of lignin in microwave-assisted alkali pretreated samples compared to the samples pretreated only with the microwave and alkali treated samples. The lignin loss was as a result of structural separation and increase in the disruption of the recalcitrant structures initiated between the biomass and alkali solution in the presence of the microwave heating. The lignin removal during pretreatment is important because it can effectively prevent the cellulase enzymes from hydrolyzing the cellulose (Kumar et al. 2009; Taherzadeh and Karimi 2008). The stronger alkali pretreatment in combination with long microwave heating and soaking time caused more solubilization of cellulose, hemicellulose and lignin. For both alkalis used, NaOH solution showed higher solubilization in the microwave pretreatments and alkali treatments compared to KOH solution in both samples. This is associated with the dipole interaction, rapidly oscillating electric field from microwave, and the ions and molecules from alkali solutions and the biomass (Hu and Wen, 2008; Xu 2015). Comparative result was found in Kashaninejad and Tabil (2011) studies. The results indicated that NaOH solution showed higher dipole moments more than $Ca(OH)_2$ used in microwave-chemical pretreatment of wheat and barley straws. Keshwani and Cheng (2010) and Zhu et al. (2006a and 2005) reported similar results with different feedstocks. The results from this study support the previous research findings reported by Tomas-Pejo et al. (2011) that identified NaOH solution as the most effective alkali reagent used in microwave pretreatment method. In general, increasing the alkali concentration with longer microwave heating time increased the cellulose content whereas decrease in alkali concentration and microwave heating time resulted in lower lignin content. But in alkali treatment, the cellulose

content increased with decreasing alkali concentration and lower soaking time whereas the hemicellulose and lignin contents decreased with higher soaking time and alkali concentration.

Biomass feedstock is bulky and has high moisture content, irregular shape, size, and low bulk density. These factors make biomass difficult to handle, transport, store and utilize in its original form. Therefore, to overcome these short-comings, pretreatment and preprocessing of the biomass makes it suitable for densification. Densification of biomass makes it economical and easy to handle, store and transport for use in pellet and biofuel industries as well as power generating stations (Adapa et al. 2009). Microwave-assisted alkali pretreatment disintegrated and disrupted the structural linkages between lignin and polysaccharides, thereby activating intermolecular bonds within the contact area of the samples and improving interlocking and binding characteristics of the particles during pelleting (Iroba et al. 2014). Pellet density, dimensional stability and tensile strength were important parameters used to evaluate the pellet quality produced. It was observed from the data that alkali concentration and microwave heating time are important factors for evaluating the physical characteristics of the pellets.

The effectiveness of microwave-assisted alkali pretreatment, microwave pretreatment and alkali treatment were further evaluated by enzymatic saccharification to ascertain the glucose yield. The optimum yield of cellulose from microwave-assisted alkali pretreatments and alkali treatments samples were selected and combined with the mixtures of cellulase and β-glucosidase enzymes for enzymatic digestion. After the saccharification, glucose in the samples was analyzed and quantified. The average available glucose yield that was released during enzymatic saccharification ranged from 2.26 to 110.05 mg/g dry sample for microwave pretreated samples and 11.56 to 88.08 mg/g dry sample for alkali treated samples, depending on the treatment combination. In general, it was observed that increasing the alkali concentration and longer microwave heating and soaking time showed higher glucose yield (Keshwani and Cheng 2010). The porous nature of the pretreated and treated biomass was susceptible to enzymatic saccharification (Zhao et al. 2010; Keshwani and Cheng 2010). The enzymatic saccharification process used in this study can be employed by the biofuel industry in quantifying the glucose yield in biomass.

The SEM analysis indicated more significant modification in the structure of biomass samples subjected to microwave-assisted alkali pretreatment compared to samples treated with

microwave only, alkali treated and untreated biomass samples. The effect of the microwave-assisted alkali pretreatment was evident in the detached fibers, collapsed cell walls, and with porous formation on the individual cell wall transverse plane surfaces compared to the microwave alone, alkali treated and untreated samples.

The interaction of alkali concentration and microwave heating time among the pellet density, dimensional stability and tensile strength significantly influenced the pellet quality of the samples regardless of the alkaline concentration and microwave heating time of the samples.

5.2 Achievement of Research Objectives

The objectives listed in Section 1.2 Chapter have been achieved over the course of the research project. They are listed from 1 to 3 below, while the thesis chapters in which they were achieved are indicated in the brackets.

The main objective of this research study was to investigate the effect of microwave-assisted alkali pretreatment of canola straw and oat hull and the subsequent densification on their enzymatic digestibility for conversion into bioethanol. The following specific objectives were achieved. The evaluation of the effect of microwave-assisted alkali pretreatment in the disintegration and disruption of lignocellulosic biomass derived from canola straw and oat hull (was elucidated in Chapters 3 and 4). The densification of the microwave-assisted alkali pretreated biomass and the evaluation of its pelletability and other physico-chemical properties (is shown in Chapter 3). The extent of digestibility of cellulose and hemicellulose during enzymatic saccharification of pretreated and untreated biomass in (Chapter 4).

5.3 Contribution to Knowledge Advancement

This thesis research study has contributed additional knowledge to the existing information on microwave-assisted alkali pretreatment technology and enzymatic hydrolysis using particularly canola straw and oat hull biomass. Results from the pellet properties indicate that ground canola straw and oat hull of 1.6 mm screen size gave a better physical quality pellets and high glucose yield. The effect of microwave-assisted alkali pretreatment of canola straw and oat hull improved enzymatic saccharification on canola straw and oat hull compared to microwave pretreatment and alkali treatment. Experimental approach used to produce single

pellets can be adopted for large scale pellet production. The research study methodology used was a follow up investigation initiated by Kashaninejad and Tabil (2011) and the study has been published in a peer review journals. This is indicative of the feasibility of the research.

5.4 Differences between Canola Straw and Oat Hull

During the study, some differences between canola straw and oat hull were observed. The major differences observed were in the alkali concentration and microwave heating time for optimum pretreatment conditions that improved the physical quality of the pellets and enzymatic digestibility. Canola straw and oat hull treated with 1.5% (w/v) NaOH and KOH at longer microwave heating time resulted in higher cellulose but decreased hemicellulose and lignin contents. However, microwave-assisted alkali pretreated canola straws produced lower hemicellulose and lignin content compared to oat hull samples. This shows that alkali solutions with microwave pretreatment caused swelling and disrupted the lignin structure on the canola straw samples.

Another difference observed between canola straw and oat hull is in the overall pellet physical characteristics. Ground 1.6 mm screen sized canola straw pellets showed better pellet properties than oat hull pellets. Microwave-assisted alkali pretreatment of canola straw resulted in high lignin content which increased particle adhesion, activated the intermolecular bonds within the contact area of canola straw sample, and improved mechanical interlocking of the particles during pelleting.

Furthermore, glucose yields increased with increasing alkali concentrations and longer microwave heating time for pretreated samples. In general, the optimum pretreatment conditions that improved enzyme digestion were 1.5% (w/v) alkali concentration with 18 min microwave heating time for canola straw whereas 0.75% (w/v) and 1.5% (w/v) alkali concentration with 6 and 18 min microwave heating time were for oat hull sample. For canola straw (1.6 and 3.2 mm), glucose yields increased from 9.45 to 110.05 mg/g and oat hull glucose yields increased from 2.26 to 97.53 mg/g. Microwave pretreatment and alkali treatment using NaOH solution at different concentrations resulted in higher glucose yields compared to KOH in both feedstocks. The canola straw showed higher glucose yields in both microwave pretreated and alkali treated.

5.5 References

1. Adapa, P., L. Tabil, G. Schoenau, 2009. Compaction characteristics of barley, canola, oat and wheat straw. *Biosystems Engineering* 104: 335 – 344.

2. Diaz, A.B., M.M. Moretti, C. Bezerra-Bussoli, C.daC.C Nunes, A. Blandino, R. da Silva and E. Gomes. 2015. Evaluation of microwave-assisted pretreatment of lignocellulosic biomass immersed in alkaline glycerol for fermentable sugars. *Bioresource Technology* 185: 316 – 323.

3. Hu, Z. and Z. Wen. 2008. Enhancing enzymatic digestibility of switchgrass by microwave assisted alkali pretreatment. *Biochemical Engineering Journal* 38: 369 – 378.

4. Iroba, K.L., L.G. Tabil, S. Sokhansanj and V. Meda. 2014. Producing durable pellets from barley straw subjected to radio frequency-alkaline and steam explosion pretreatments. *International Journal of Agricultural and Biological Eng*ineering 7(3): 68 - 82.

5. Kashaninejad, M. and L. G. Tabil. 2011. Effect of microwave: chemical pretreatment on compression characteristics of biomass grinds. *Biosystems Engineering* 108: 36 – 45.

6. Keshwani, D.R. and J.J. Cheng. 2010. Microwave-based alkali pretreatment of switchgrass and coastal bermudagrass for bioethanol production. *Biotechnology Progress* 26(3): 644 – 652.

7. Kumar, P., D.M. Barrett, M.J. Delwiche and P. Stroeve. 2009. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Industrial and Engineering Chemistry Research* 48: 3713 – 3729.

8. Ma, H., W. Liu, X. Chen, Y. Wu and Z. Yu. 2009. Enhanced enzymatic saccharification of rice straw by microwave pretreatment. *Bioresource Technology* 100: 1279 – 1284.

9. Nomanbhay, S.M., R. Hussain and K. Palanisamy. 2013. Microwave-assisted alkaline pretreatment and microwave assisted enzymatic saccharification of oil palm empty fruit bunch fiber foe enhance fermentable sugar yield. *Journal of Sustainable Bioenergy System* 3: 7 – 17.

10. Taherzadeh, M.J. and K. Karimi. 2008. Pretreatment of lignocellulosic waste to improve ethanol and biogas production: a review. *International Journal of Molecular Sciences* 9: 1621 – 1651.

11. Tomas-Pejo, E., P. Alvria, M. Ballesteros and M.J. Negro. 2011. Pretreatment technologies for lignocellulose to bioethanol conversion. In *Biofuels: Alternative Feedstocks and Conversion Processes,* ed. A. Pandey E. Gnansounou, C.G. Dussap, C. Larroche and S.C. Ricke, 149 – 177. Oxford, UK Academic Press.

12. Xu, J. 2015. Microwave pretreatment. In *Pretreatment of Biomass: Processes and Technologies*, ed. A. Pandey, 157 – 172. Waltham, MA: Elsevier.

13. Zhao, X., Y. Zhou, G. Zheng and D. Liu. 2010. Microwave pretreatment of substrates for cellulase production by solid-state fermentation. *Applied Biochemistry and Biotechnology* 160: 1557 – 71.

14. Zhu, S., Y. Wu, Z. Yu, Q. Chen, G. Wu, F. Yu, C. Wang and S. Jin. 2006a. Microwave-assisted alkali pretreatment of wheat straw and its enzymatic hydrolysis. *Biosystems Engineering* 93(3): 437 – 442.

15. Zhu, S., Y. Wu, Z. Yu, J. Liao and Y. Zhang. 2005. Pretreatment by microwave/alkali of rice straw and its enzymatic hydrolysis. *Process Biochemistry* 40: 3082 – 3086.

Chapter 6

Conclusions and Recommendations

This chapter presents the overall project conclusions and provides recommendations for future studies.

6.1 Project Conclusions

The following conclusions are made based on the experiments and analysis performed in the course of this research:

1.   The application of microwave-assisted alkali pretreatment to disrupt, disintegrate and breakdown the structural cell walls of lignocellulosic biomass was investigated. The following are the effects of the microwave-assisted alkali pretreatment on the biomass:

    - The biomass matrix in the lignin was disrupted and disintegrated, which improved accessibility and digestibility of cellulose and hemicellulose.

    - Increasing alkali concentration and long microwave heating time in microwave-assisted alkali pretreatment resulted to more solubilization of cellulose, hemicellulose and lignin contents compared to microwave pretreatment and alkali treatment.

    - Alkalis (NaOH and KOH) solutions used in the microwave pretreatments and alkali treatments caused swelling and lignin structure disruption in the biomass that resulted to solubility of lignin in the feedstocks.

    - In general, high alkali concentration with long microwave heating time resulted in high physical properties and pellet characterizations, which significantly affected the physical qualities of canola straw and oat hull pellets produced.

2.   Ground samples pretreated by microwave-assisted alkali had significantly higher ash content, bulk and particle densities (especially biomass samples pretreated with microwave/NaOH) than samples pretreated with microwave alone and untreated samples.

3.   Microwave-assisted alkali pretreatment of canola straw and oat hull resulted in better physical quality pellets characteristics (tensile strength, dimensional stability and pellet density), which could be used as a potential feedstock in thermochemical process for

bioenergy industry. Thus, alkali concentration of 1.5% with a microwave power level 713 W and microwave heating of approximately 6 min resulted in high tensile strength of canola straw pellets whereas a microwave heating time of 9 – 18 min resulted in high tensile strength of oat hull pellets.

4.   Microwave-assisted alkali pretreatment of canola straw and oat hull gave a high glucose yield compared to and alkali treatment during enzymatic saccharification. The optimum microwave-assisted alkali pretreatment conditions that gave the high glucose yield for both substrates were ground in 1.6 mm screen hammer mill with 1.5% and 0.75 % NaOH/18 min.

5.   Overall, microwave-assisted alkali pretreatment on canola straw and oat hull improved biomass pellet quality and glucose (sugar) yield for bioethanol production. Furthermore, microwave/NaOH pretreatment resulted in better physical quality pellets from both canola straw and oat hull samples than microwave/KOH pretreatment.

6.2 Project Recommendations

The following recommendations are made for future investigations:

- Investigate the energy analysis and economic cost requirements of microwave-assisted alkali pretreatment and enzymatic saccharification technique for industrial scale up.
- Design a continuous microwave-assisted pretreatment process that would remove all the challenges associated with microwave heating for easy pelleting (lab or Pilot-scale).
- Design a microwave-assisted alkali pretreatment reactor at lower power 200 – 400 W with residence time not more than 25 min in order to obtain the optimal pretreatment condition that will improve enzymatic digestibility for bioethanol production.

A. Particle Size Analysis of Ground Canola Straw and Oat Hull.

Table A. 1. Geometric mean diameter and geometric standard deviation of ground canola straw and oat hull 1.6 mm screen size.

Hammer mill screen size (mm)	Canola straw	Oat hulls
> 841 μm	10.00	5.91
595 - 841 μm	26.66	21.65
420 - 595μm	24.28	35.58
297 - 420 μm	15.17	21.98
210 - 297 μm	10.34	8.26
149 - 210 μm	6.34	3.30
< 149 μm	7.21	3.32
d_{gw} (mm)	0.348	0.370
S_{gw} (mm)	0.280	0.217
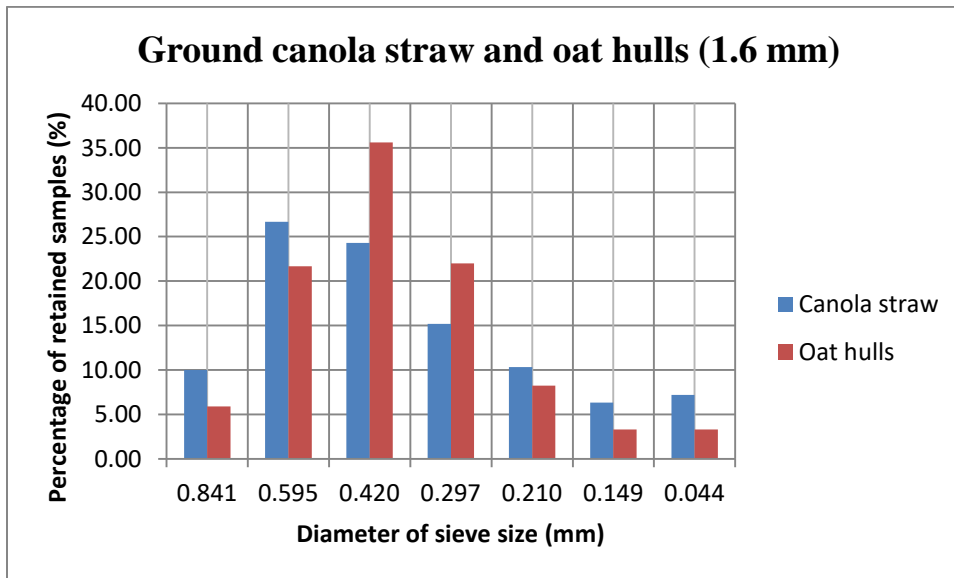
Figure A. 1. Mass retained over sieves representing particle size distribution of ground canola straw and oat hull (1.6 mm).

Table A. 2. Geometric mean diameter and geometric standard deviation of ground canola straw and oat hull 3.2 mm screen size.

Hammer mill screen size (mm)	Canola straw	Oat hull
> 2.00 mm	0.31	0.00
1.41 - 2.00 mm	7.47	1.64
1.19 - 1.41 mm	13.73	4.36
1.00 - 1.19 mm	10.20	4.43
841μm - 1.00 mm	10.98	15.13
595 - 841 μm	18.34	40.21
420 - 595 μm	12.88	22.16
297 - 420 μm	8.24	6.40
210 - 297 μm	6.31	2.56
149 - 210 μm	4.71	1.33
105 - 149 μm	1.29	0.69
74 - 105 μm	1.86	0.56
< 74 μm	3.67	0.53
d_{gw} (mm)	0.520	0.547
S_{gw} (mm)	0.498	0.284
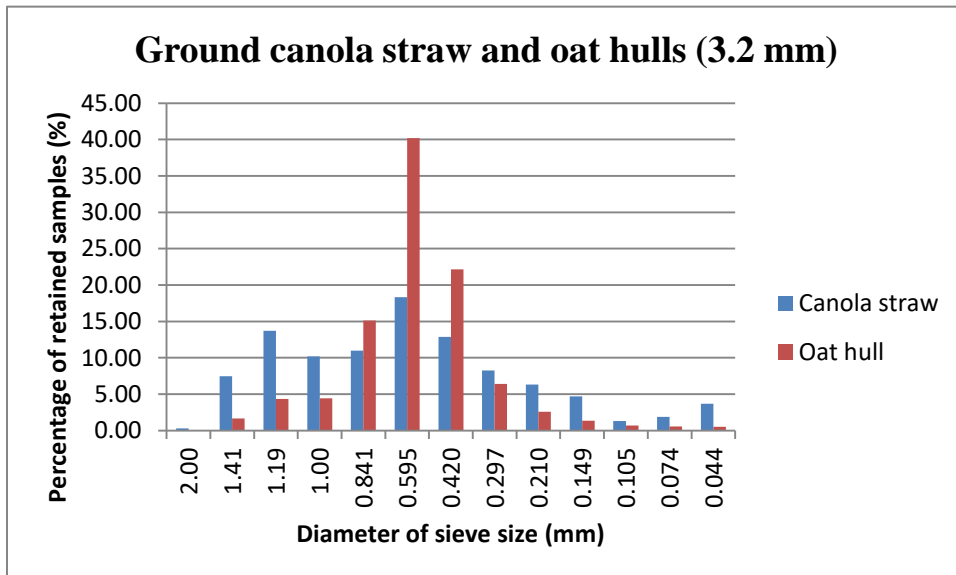
Figure A. 2. Mass retained over sieves representing particle size distribution of ground canola straw and oat hull (3.2 mm).

Appendix B

B. ANOVA Table for Response Surface Quadratic Models of Microwave-Assisted Alkali Pretreated Ground Canola Straw.

Table B. 1. Ash content quadratic model of microwave/NaOH pretreated canola straw ground with 1.6 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	410.85	5	82.17	90.97	0.0018
A- Alkali conc.	404.92	1	404.92	448.29	0.0002
B- MW heating time	0.65	1	0.65	0.72	0.4575
AB	1.58	1	1.58	1.74	0.2784
A^2	3.64	1	3.64	4.03	0.1385
B^2	0.067	1	0.067	0.074	0.8027
Residual	2.71	3	0.9		
Cor. Total	413.56	8			

Std. Dev.	0.95	**R-Squared**	0.9934	
Mean	13.99	**Adj. R-Squared**	0.9825	
C.V. %	6.79	**Pred. R-Squared**	0.9209	
PRESS	32.69	**Adeq. Precision**	22.79	

Table B. 2. Bulk density quadratic model of microwave/NaOH pretreated canola straw ground with 1.6 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	13583.7	5	2716.74	21.83	0.0145
A- Alkali conc.	8090.88	1	8090.88	65.01	0.004
B- MW heating time	3696.19	1	3696.19	29.7	0.0121
AB	1789.29	1	1789.29	14.38	0.0322
A^2	0.14	1	0.14	1.10E-03	0.9756
B^2	7.19	1	7.19	0.058	0.8255
Residual	373.36	3	124.45		
Cor. Total	13957.06	8			

Std. Dev.	11.16	R-Squared	0.9732	
Mean	168.21	Adj. R-Squared	0.9287	
C.V. %	6.63	Pred. R-Squared	0.6913	
PRESS	4308.34	Adeq. Precision	13.513	

Table B. 3. Particle density quadratic model of microwave/NaOH pretreated canola straw ground with 1.6 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	1.77E+05	5	35380.98	248.34	0.0004
A- Alkali conc.	1.09E+05	1	1.09E+05	768.09	0.0001
B- MW heating time	52856.32	1	52856.32	371	0.0003
AB	1414.14	1	1414.14	9.93	0.0512
A^2	12942.8	1	12942.8	90.85	0.0024
B^2	263.35	1	263.35	1.85	0.2671
Residual	427.4	3	142.47		
Cor. Total	1.77E+05	8			

Std. Dev.	11.94	R-Squared	0.9976	
Mean	1359.85	Adj. R-Squared	0.9936	
C.V. %	0.88	Pred. R-Squared	0.9709	
PRESS	5169.12	Adeq. Precision	46.976	

Table B. 4. Ash content quadratic model of microwave/KOH pretreated canola straw ground with 1.6 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	306.24	5	61.25	658.86	< 0.0001
A- Alkali conc.	306.16	1	306.16	3293.52	< 0.0001
B- MW heating time	0.017	1	0.017	0.18	0.6972
AB	8.10E-03	1	8.10E-03	0.087	0.7871
A^2	0.041	1	0.041	0.44	0.5537
B^2	8.02E-03	1	8.02E-03	0.086	0.7881
Residual	0.28	3	0.093		
Cor. Total	306.52	8			

Std. Dev.	0.3	**R-Squared**	0.9991	
Mean	12.51	**Adj. R-Squared**	0.9976	
C.V. %	2.44	**Pred. R-Squared**	0.9889	
PRESS	3.4	**Adeq. Precision**	57.818	

Table B. 5. Bulk density quadratic model of microwave/KOH pretreated canola straw ground with 1.6 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	4439.83	5	887.97	26.3	0.0111
A- Alkali conc.	2628.39	1	2628.39	77.86	0.0031
B- MW heating time	1354.8	1	1354.8	40.13	0.008
AB	407.43	1	407.43	12.07	0.0402
A^2	15.2	1	15.2	0.45	0.5503
B^2	34	1	34	1.01	0.3895
Residual	101.28	3	33.76		
Cor. Total	4541.11	8			

Std. Dev.	5.81	**R-Squared**	0.9777	
Mean	151.58	**Adj. R-Squared**	0.9405	
C.V. %	3.83	**Pred. R-Squared**	0.7371	
PRESS	1193.89	**Adeq. Precision**	15.158	

Table B. 6. Particle density quadratic model of microwave/KOH pretreated canola straw ground with 1.6 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	1.29E+05	5	25724.97	4.83	0.1124
A- Alkali conc.	71945.88	1	71945.88	13.52	0.0348
B- MW heating time	35394.05	1	35394.05	6.65	0.0818
AB	1258.12	1	1258.12	0.24	0.6601
A^2	204.42	1	204.42	0.038	0.8571
B^2	19822.4	1	19822.4	3.73	0.1491
Residual	15962.86	3	5320.95		
Cor. Total	1.45E+05	8			

Std. Dev.	72.94	R-Squared	0.8896
Mean	1310.86	Adj. R-Squared	0.7056
C.V. %	5.56	Pred. R-Squared	-0.1969
PRESS	1.73E+05	Adeq. Precision	6.936

Table B. 7. Ash content quadratic model of microwave/NaOH pretreated canola straw ground with 3.2 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	419.3	5	83.86	237.36	0.0004
A- Alkali conc.	416.67	1	416.67	1179.34	< 0.0001
B- MW heating time	0.11	1	0.11	0.32	0.6126
AB	0.25	1	0.25	0.71	0.462
A^2	2.23	1	2.23	6.32	0.0866
B^2	0.037	1	0.037	0.11	0.7664
Residual	1.06	3	0.35		
Cor. Total	420.36	8			

Std. Dev.	0.59	R-Squared	0.9975
Mean	14.02	Adj. R-Squared	0.9933
C.V. %	4.24	Pred. R-Squared	0.9698
PRESS	12.7	Adeq. Precision	35.372

Table B. 8. Bulk density quadratic model of microwave/NaOH pretreated canola straw ground with 3.2 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	14445.65	5	2889.13	39.5	0.0061
A- Alkali conc.	8970.67	1	8970.67	122.64	0.0016
B- MW heating time	3865.88	1	3865.88	52.85	0.0054
AB	1436.79	1	1436.79	19.64	0.0213
A^2	65.44	1	65.44	0.89	0.414
B^2	106.87	1	106.87	1.46	0.3133
Residual	219.44	3	73.15		
Cor. Total	14665.09	8			

Std. Dev.	8.55	R-Squared	0.985	
Mean	153.91	Adj. R-Squared	0.9601	
C.V. %	5.56	Pred. R-Squared	0.8236	
PRESS	2586.39	Adeq. Precision	18.47	

Table B. 9. Particle density quadratic model of microwave/NaOH pretreated canola straw ground with 3.2 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	2.87E+05	5	57352.41	42.8	0.0055
A- Alkali conc.	2.27E+05	1	2.27E+05	169.01	0.001
B- MW heating time	16149.21	1	16149.21	12.05	0.0403
AB	3249	1	3249	2.42	0.2173
A^2	24813.24	1	24813.24	18.52	0.0231
B^2	16070.66	1	16070.66	11.99	0.0405
Residual	4020.03	3	1340.01		
Cor. Total	2.91E+05	8			

Std. Dev.	36.61	R-Squared	0.9862	
Mean	1251.77	Adj. R-Squared	0.9631	
C.V. %	2.92	Pred. R-Squared	0.8835	
PRESS	33886.41	Adeq. Precision	16.782	

Table B. 10. Ash content quadratic model of microwave/KOH pretreated canola straw ground with 3.2 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	342.66	5	68.53	728.32	< 0.0001
A- Alkali conc.	342.62	1	342.62	3641.19	< 0.0001
B- MW heating time	0.018	1	0.018	0.19	0.6902
AB	7.23E-03	1	7.23E-03	0.077	0.7997
A^2	6.42E-03	1	6.42E-03	0.068	0.8108
B^2	5.34E-03	1	5.34E-03	0.057	0.8271
Residual	0.28	3	0.094		
Cor. Total	342.94	8			

Std. Dev.	0.31	R-Squared	0.9992	
Mean	12.91	Adj. R-Squared	0.9978	
C.V. %	2.38	Pred. R-Squared	0.99	
PRESS	3.44	Adeq. Precision	60.781	

Table B. 11. Bulk density quadratic model of microwave/KOH pretreated canola straw ground with 3.2 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	5422.96	5	1084.59	19.61	0.0169
A- Alkali conc.	2693.67	1	2693.67	48.71	0.006
B- MW heating time	1931.42	1	1931.42	34.93	0.0097
AB	561.45	1	561.45	10.15	0.0498
A^2	195.16	1	195.16	3.53	0.1569
B^2	41.25	1	41.25	0.75	0.4513
Residual	165.89	3	55.3		
Cor. Total	5588.85	8			

Std. Dev.	7.44	R-Squared	0.9703	
Mean	135.05	Adj. R-Squared	0.9208	
C.V. %	5.51	Pred. R-Squared	0.6433	
PRESS	1993.71	Adeq. Precision	12.978	

Table B. 12. Particle density quadratic model of microwave/KOH pretreated canola straw ground with 3.2 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	2.89E+05	5	57854.2	12.43	0.0322
A- Alkali conc.	2.25E+05	1	2.25E+05	48.33	0.0061
B- MW heating time	32462.03	1	32462.03	6.98	0.0776
AB	2342.08	1	2342.08	0.5	0.5292
A^2	115.77	1	115.77	0.025	0.8847
B^2	29445.98	1	29445.98	6.33	0.0865
Residual	13961	3	4653.67		
Cor. Total	3.03E+05	8			

Std. Dev.	68.22	R-Squared	0.954	
Mean	1216.5	Adj. R-Squared	0.8772	
C.V. %	5.61	Pred. R-Squared	0.4768	
PRESS	1.59E+05	Adeq. Precision	10.016	

Appendix C

C. ANOVA Table for Response Surface Quadratic Models of Microwave-Assisted Alkali Pretreated Ground Oat Hull.

Table C. 1. Ash content quadratic model of microwave/NaOH pretreated oat hull ground with 1.6 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	181.06	5	36.21	457.02	0.0002
A- Alkali conc.	177.89	1	177.89	2245.04	< 0.0001
B- MW heating time	1.18	1	1.18	14.88	0.0308
AB	0.11	1	0.11	1.42	0.3196
A^2	1.79	1	1.79	22.54	0.0177
B^2	0.097	1	0.097	1.22	0.3497
Residual	0.24	3	0.079		
Cor. Total	181.3	8			

Std. Dev.	0.28	R-Squared	0.9987	
Mean	9.96	Adj. R-Squared	0.9965	
C.V. %	2.83	Pred. R-Squared	0.9861	
PRESS	2.51	Adeq. Precision	51.24	

Table C. 2. Bulk density quadratic model of microwave/NaOH pretreated oat hull ground with 1.6 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	12555.15	5	2511.03	9.23	0.0484
A- Alkali conc.	2336.03	1	2336.03	8.58	0.061
B- MW heating time	9143.17	1	9143.17	33.6	0.0102
AB	23.67	1	23.67	0.087	0.7873
A^2	884.1	1	884.10	3.25	0.1693
B^2	168.18	1	168.18	0.62	0.4892
Residual	816.34	3	272.11		
Cor. Total	13371.49	8			

Std. Dev.	16.5	R-Squared	0.9389	
Mean	294.19	Adj. R-Squared	0.8372	
C.V. %	5.61	Pred. R-Squared	0.2682	
PRESS	9784.89	Adeq. Precision	9.003	

Table C. 3. Particle density quadratic model of microwave/NaOH pretreated oat hull ground with 1.6 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	25052.94	5	5010.59	22.02	0.0143
A- Alkali conc.	24420.09	1	24420.09	107.34	0.0019
B- MW heating time	99.80	1	99.80	0.44	0.5551
AB	121.22	1	121.22	0.53	0.5183
A^2	29.39	1	29.39	0.13	0.7431
B^2	382.45	1	382.45	1.68	0.2855
Residual	682.50	3	227.50		
Cor. Total	25735.44	8			

Std. Dev.	15.08	R-Squared	0.9735	
Mean	1487.76	Adj. R-Squared	0.9293	
C.V. %	1.01	Pred. R-Squared	0.6814	
PRESS	8198.52	Adeq. Precision	11.599	

Table C. 4. Ash content quadratic model of microwave/KOH pretreated oat hull ground with 1.6 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	111.82	5	22.36	865.65	< 0.0001
A- Alkali conc.	105.59	1	105.59	4086.84	< 0.0001
B- MW heating time	0.46	1	0.46	17.78	0.0244
AB	7.23E-03	1	7.23E-03	0.28	0.6336
A^2	5.75	1	5.75	222.4	0.0007
B^2	0.024	1	0.024	0.94	0.4045
Residual	0.078	3	0.026		
Cor. Total	111.9	8			

Std. Dev.	0.16	R-Squared	0.9993	
Mean	8.46	Adj. R-Squared	0.9982	
C.V. %	1.9	Pred. R-Squared	0.9923	
PRESS	0.86	Adeq. Precision	68.145	

Table C. 5. Bulk density quadratic model of microwave/KOH pretreated oat hull ground with 1.6 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	8285.24	5	1657.05	10.35	0.0414
A- Alkali conc.	169.5	1	169.50	1.06	0.3793
B- MW heating time	7401.19	1	7401.19	46.21	0.0065
AB	209.24	1	209.24	1.31	0.336
A^2	391.16	1	391.16	2.44	0.216
B^2	114.16	1	114.16	0.71	0.4605
Residual	480.45	3	160.15		
Cor. Total	8765.68	8			

Std. Dev.	12.66	R-Squared	0.9452	
Mean	282.12	Adj. R-Squared	0.8538	
C.V. %	4.49	Pred. R-Squared	0.4204	
PRESS	5080.46	Adeq. Precision	9.366	

Table C. 6. Particle density quadratic model of microwave/KOH pretreated oat hull ground with 1.6 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	16612.09	5	3322.42	16.47	0.0217
A- Alkali conc.	15026.01	1	15026.01	74.47	0.0033
B- MW heating time	96.72	1	96.72	0.48	0.5385
AB	440.58	1	440.58	2.18	0.236
A^2	660.78	1	660.78	3.28	0.168
B^2	388	1	388.00	1.92	0.2596
Residual	605.3	3	201.77		
Cor. Total	17217.39	8			

Std. Dev.	14.2	R-Squared	0.9648	
Mean	1466.67	Adj. R-Squared	0.9063	
C.V. %	0.97	Pred. R-Squared	0.6674	
PRESS	5725.72	Adeq. Precision	10.44	

Table C. 7. Ash content quadratic model of microwave/NaOH pretreated oat hull ground with 3.2 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	175.9	5	35.18	1246.91	< 0.0001
A- Alkali conc.	172.48	1	172.48	6113.47	< 0.0001
B- MW heating time	1.04	1	1.04	36.92	0.0089
AB	7.23E-03	1	7.23E-03	0.26	0.6477
A^2	2.35	1	2.35	83.45	0.0028
B^2	0.013	1	0.013	0.45	0.5488
Residual	0.085	3	0.028		
Cor. Total	175.99	8			

Std. Dev.	0.17	R-Squared	0.9995	
Mean	9.83	Adj. R-Squared	0.9987	
C.V. %	1.71	Pred. R-Squared	0.9943	
PRESS	1.00	Adeq. Precision	84.265	

Table C. 8. Bulk density quadratic model of microwave/NaOH pretreated oat hull ground with 3.2 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	11234.7	5	2246.94	2.13	0.2831
A- Alkali conc.	6822.23	1	6822.23	6.48	0.0843
B- MW heating time	2681.4	1	2681.4	2.54	0.2089
AB	43.89	1	43.89	0.042	0.8513
A^2	671.98	1	671.98	0.64	0.4829
B^2	1015.2	1	1015.2	0.96	0.3987
Residual	3160.86	3	1053.62		
Cor. Total	14395.55	8			

Std. Dev.	32.46	R-Squared	0.7804	
Mean	245.63	Adj. R-Squared	0.4145	
C.V. %	13.21	Pred. R-Squared	-1.61	
PRESS	37572	Adeq. Precision	4.14	

Table C. 9. Particle density quadratic model of microwave/NaOH pretreated oat hull ground with 3.2 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	85540.12	5	17108.02	3.51	0.1654
A- Alkali conc.	43596.85	1	43596.85	8.93	0.0582
B- MW heating time	571.35	1	571.35	0.12	0.7548
AB	7.76	1	7.76	1.59E-03	0.9707
A^2	36927.65	1	36927.65	7.57	0.0707
B^2	4436.51	1	4436.51	0.91	0.4107
Residual	14642.52	3	4880.84		
Cor. Total	1.00E+05	8			

Std. Dev.	69.86	R-Squared	0.8538	
Mean	1416.61	Adj. R-Squared	0.6102	
C.V. %	4.93	Pred. R-Squared	-0.3174	
PRESS	1.32E+05	Adeq. Precision	4.873	

Table C. 10. Ash content quadratic model of microwave/KOH pretreated oat hull ground with 3.2 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	121.91	5	24.38	1572.71	< 0.0001
A- Alkali conc.	115.54	1	115.54	7453.17	< 0.0001
B- MW heating time	0.78	1	0.78	50.16	0.0058
AB	0.027	1	0.027	1.76	0.277
A^2	5.54	1	5.54	357.64	0.0003
B^2	0.013	1	0.013	0.83	0.4305
Residual	0.047	3	0.016		
Cor. Total	121.95	8			

Std. Dev.	0.12	R-Squared	0.9996	
Mean	8.67	Adj. R-Squared	0.999	
C.V. %	1.44	Pred. R-Squared	0.9954	
PRESS	0.57	Adeq. Precision	93.414	

Table C. 11. Bulk density quadratic model of microwave/KOH pretreated oat hull ground with 3.2 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	3227.11	5	645.42	4.28	0.1305
A- Alkali conc.	1177.68	1	1177.68	7.81	0.0681
B- MW heating time	1893.22	1	1893.22	12.56	0.0383
AB	3.61	1	3.61	0.024	0.8868
A^2	134.48	1	134.48	0.89	0.4146
B^2	18.12	1	18.12	0.12	0.7517
Residual	452.23	3	150.74		
Cor. Total	3679.33	8			

Std. Dev.	12.28	R-Squared	0.8771	
Mean	229.3	Adj. R-Squared	0.6722	
C.V. %	5.35	Pred. R-Squared	-0.4449	
PRESS	5316.14	Adeq. Precision	6.339	

Table C. 12. Particle density quadratic model of microwave/KOH pretreated oat hull ground with 3.2 mm screen size hammer mill.

Source	Sum of squares	df	Mean square	F value	p-value Prob. > F
Model	34202.36	5	6840.47	23.81	0.0128
A- Alkali conc.	33729	1	33729	117.41	0.0017
B- MW heating time	338.25	1	338.25	1.18	0.3573
AB	1.28	1	1.28	4.45E-03	0.951
A^2	123.14	1	123.14	0.43	0.5594
B^2	10.69	1	10.69	0.037	0.8594
Residual	861.85	3	287.28		
Cor. Total	35064.21	8			

Std. Dev.	16.95	R-Squared	0.9754	
Mean	1454.26	Adj. R-Squared	0.9345	
C.V. %	1.17	Pred. R-Squared	0.704	
PRESS	10377.47	Adeq. Precision	11.921	

Appendix D

D. ANOVA Table for Response Surface Quadratic Models of Microwave-Assisted Alkali and Microwave Alone Pretreated Canola Straw Pellets.

Table D. 1. Pellet density quadratic model of microwave/NaOH and microwave alone pretreated 1.6 mm screen size hammer mill canola straw pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	1.52E+05	5	30371.91	48.13	0.0046
A- Alkali conc.	1.33E+05	1	1.33E+05	210.41	0.0007
B- MW heating time	168.65	1	168.65	0.27	0.6409
AB	2283.88	1	2283.88	3.62	0.1533
A^2	16560.79	1	16560.79	26.25	0.0144
B^2	74.95	1	74.95	0.12	0.7531
Residual	1893	3	631		
Cor. Total	1.54E+05	8			

Std. Dev.	25.12	R-Squared	0.9877	
Mean	1220.73	Adj. R-Squared	0.9672	
C.V. %	2.06	Pred. R-Squared	0.8895	
PRESS	16982.63	Adeq. Precision	16.836	

Table D. 2. Dimensional stability quadratic model of microwave/NaOH and microwave alone pretreated 1.6 mm screen size hammer mill canola straw pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	17.144	5	3.428800556	4.081442	0.1382
A- Alkali conc.	5.920267	1	5.920266667	7.047136	0.0767
B- MW heating time	4.842017	1	4.842016667	5.763651	0.0958
AB	0.366025	1	0.366025	0.435695	0.5564
A^2	2.361689	1	2.361688889	2.811215	0.1922
B^2	3.654006	1	3.654005556	4.349513	0.1283
Residual	2.520286	3	0.84009537		
Cor. Total	19.66429	8			

Std. Dev.	0.916567	R-Squared	0.871834	
Mean	0.381111	Adj. R-Squared	0.658225	
C.V. %	240.4987	Pred. R-Squared	-0.05554	
PRESS	20.75635	Adeq. Precision	6.190087	

Table D. 3. Tensile strength quadratic model of microwave/NaOH and microwave alone pretreated 1.6 mm screen size hammer mill canola straw pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	22.95	5	4.59	17.19	0.0204
A- Alkali conc.	13.35	1	13.35	50	0.0058
B- MW heating time	5.98	1	5.98	22.4	0.0179
AB	1.89	1	1.89	7.08	0.0763
A^2	1.58	1	1.58	5.91	0.0932
B^2	0.15	1	0.15	0.55	0.5111
Residual	0.8	3	0.27		
Cor. Total	23.75	8			

Std. Dev.	0.52	**R-Squared**	0.9663	
Mean	2.46	**Adj. R-Squared**	0.9101	
C.V. %	20.99	**Pred. R-Squared**	0.6091	
PRESS	9.28	**Adeq. Precision**	11.804	

Table D. 4. Pellet density quadratic model of microwave/KOH and microwave alone pretreated 1.6 mm screen size hammer mill canola straw pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	1.51E+05	5	30258.55	299.56	0.0003
A- Alkali conc.	1.45E+05	1	1.45E+05	1431.27	< 0.0001
B- MW heating time	6347.25	1	6347.25	62.84	0.0042
AB	135.26	1	135.26	1.34	0.331
A^2	13.69	1	13.69	0.14	0.7372
B^2	224.65	1	224.65	2.22	0.2327
Residual	303.03	3	101.01		
Cor. Total	1.52E+05	8			

Std. Dev.	10.05	**R-Squared**	0.998	
Mean	1197.74	**Adj. R-Squared**	0.9947	
C.V. %	0.84	**Pred. R-Squared**	0.9762	
PRESS	3606.72	**Adeq. Precision**	45.759	

Table D. 5. Dimensional stability quadratic model of microwave/KOH and microwave alone pretreated 1.6 mm screen size hammer mill canola straw pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	10.00574	5	2.001147222	17.21403	0.0204
A- Alkali conc.	1.421067	1	1.421066667	12.22413	0.0396
B- MW heating time	6.06015	1	6.06015	52.12991	0.0055
AB	0.198025	1	0.198025	1.703427	0.2829
A^2	1.323022	1	1.323022222	11.38075	0.0433
B^2	1.003472	1	1.003472222	8.63195	0.0606
Residual	0.348753	3	0.116250926		
Cor. Total	10.35449	8			

Std. Dev.	0.340956	R-Squared	0.966319	
Mean	0.978889	Adj. R-Squared	0.910183	
C.V. %	34.83091	Pred. R-Squared	0.608047	
PRESS	4.058475	Adeq. Precision	12.68906	

Table D. 6. Tensile strength quadratic model of microwave/KOH and microwave alone pretreated 1.6 mm screen size hammer mill canola straw pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	9.61	5	1.92	15.14	0.0244
A- Alkali conc.	6.87	1	6.87	54.11	0.0052
B- MW heating time	2.18	1	2.18	17.2	0.0255
AB	0.55	1	0.55	4.31	0.1294
A^2	8.02E-03	1	8.02E-03	0.063	0.8178
B^2	2.69E-03	1	2.69E-03	0.021	0.8935
Residual	0.38	3	0.13		
Cor. Total	9.99	8			

Std. Dev.	0.36	R-Squared	0.9619	
Mean	1.76	Adj. R-Squared	0.8984	
C.V. %	20.19	Pred. R-Squared	0.5502	
PRESS	4.49	Adeq. Precision	11.504	

Table D. 7. Pellet density quadratic model of microwave/NaOH and microwave alone pretreated 3.2 mm screen size hammer mill canola straw pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	144927.2	5	28985.44309	58.48787	0.0034
A- Alkali conc.	128958.2	1	128958.1562	260.2164	0.0005
B- MW heating time	819.4691	1	819.4690667	1.653554	0.2887
AB	2305.92	1	2305.9204	4.652969	0.1199
A^2	12797.33	1	12797.33347	25.82292	0.0147
B^2	46.33636	1	46.33635556	0.093499	0.7798
Residual	1486.741	3	495.5803926		
Cor. Total	146414	8			

Std. Dev.	22.26163	R-Squared	0.989846	
Mean	1241.886	Adj. R-Squared	0.972922	
C.V. %	1.792567	Pred. R-Squared	0.881364	
PRESS	17370.03	Adeq. Precision	18.77309	

Table D. 8. Dimensional stability quadratic model of microwave/NaOH and microwave alone pretreated 3.2 mm screen size hammer mill canola straw pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	36.17	5	7.23	6.98	0.0703
A- Alkali conc.	5.41	1	5.41	5.23	0.1063
B- MW heating time	30.06	1	30.06	29.02	0.0125
AB	4.20E-01	1	4.20E-01	0.41	0.5685
A^2	8.50E-02	1	8.50E-02	8.20E-02	0.7927
B^2	0.18	1	0.18	0.18	0.7033
Residual	3.11	3	1.04		
Cor. Total	39.27	8			

Std. Dev.	1.02	R-Squared	0.9209	
Mean	0.66	Adj. R-Squared	0.789	
C.V. %	153.19	Pred. R-Squared	0.0352	
PRESS	37.89	Adeq. Precision	7.673	

Table D. 9. Tensile strength quadratic model of microwave/NaOH and microwave alone pretreated 3.2 mm screen size hammer mill canola straw pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	16.08	5	3.22	4.25	0.1316
A- Alkali conc.	10.3	1	10.3	13.61	0.0345
B- MW heating time	4.42	1	4.42	5.84	0.0944
AB	0.38	1	0.38	0.5	0.5305
A^2	0.98	1	0.98	1.3	0.3377
B^2	5.00E-05	1	5.00E-05	6.61E-05	0.994
Residual	2.27	3	0.76		
Cor. Total	18.34	8			

Std. Dev.	0.87	R-Squared	0.8763	
Mean	2.56	Adj. R-Squared	0.6701	
C.V. %	34.02	Pred. R-Squared	-0.3643	
PRESS	25.03	Adeq. Precision	6.107	

Table D. 10. Pellet density quadratic model of microwave/KOH and microwave alone pretreated 3.2 mm screen size hammer mill canola straw pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	1.31E+05	5	26235.13	54.49	0.0038
A- Alkali conc.	1.28E+05	1	1.28E+05	266.12	0.0005
B- MW heating time	2055.35	1	2055.35	4.27	0.1307
AB	266.67	1	266.67	0.55	0.5107
A^2	472.17	1	472.17	0.98	0.395
B^2	254.7	1	254.7	0.53	0.5196
Residual	1444.37	3	481.46		
Cor. Total	1.33E+05	8			

Std. Dev.	21.94	R-Squared	0.9891	
Mean	1209.63	Adj. R-Squared	0.971	
C.V. %	1.81	Pred. R-Squared	0.8821	
PRESS	15632.94	Adeq. Precision	18.432	

Table D. 11. Dimensional stability quadratic model of microwave/KOH and microwave alone pretreated 3.2 mm screen size hammer mill canola straw pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	16.06	5	3.21	8.24	0.0564
A- Alkali conc.	3.82	1	3.82	9.81	0.052
B- MW heating time	10.01	1	10.01	25.69	0.0148
AB	0.93	1	0.93	2.39	0.2199
A^2	0.038	1	0.038	0.098	0.7745
B^2	1.25	1	1.25	3.22	0.1708
Residual	1.17	3	0.39		
Cor. Total	17.23	8			

Std. Dev.	0.62	R-Squared	0.9321	
Mean	0.93	Adj. R-Squared	0.819	
C.V. %	67.04	Pred. R-Squared	0.2042	
PRESS	13.71	Adeq. Precision	8.201	

Table D. 12. Tensile strength quadratic model of microwave/KOH and microwave alone pretreated 3.2 mm screen size hammer mill canola straw pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	14.9	5	2.98	19.04	0.0176
A- Alkali conc.	11.21	1	11.21	71.63	0.0035
B- MW heating time	1.83	1	1.83	11.67	0.042
AB	0.89	1	0.89	5.71	0.0968
A^2	0.83	1	0.83	5.29	0.105
B^2	0.14	1	0.14	0.92	0.4081
Residual	0.47	3	0.16		
Cor. Total	15.37	8			

Std. Dev.	0.4	R-Squared	0.9695	
Mean	2.17	Adj. R-Squared	0.9186	
C.V. %	18.27	Pred. R-Squared	0.6483	
PRESS	5.4	Adeq. Precision	12.466	

Appendix E

E. ANOVA Table for Response Surface Quadratic Models of Microwave-Assisted Alkali and Microwave Alone Pretreated Oat Hull Pellets.

Table E. 1. Pellet density quadratic model of microwave/NaOH and microwave alone pretreated 1.6 mm screen size hammer mill oat hull pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	1.07E+05	5	21394.55	17.81	0.0194
A- Alkali conc.	88965.16	1	88965.16	74.06	0.0033
B- MW heating time	2287.35	1	2287.35	1.9	0.2615
AB	732.24	1	732.24	0.61	0.4919
A^2	14624.49	1	14624.49	12.17	0.0398
B^2	363.51	1	363.51	0.3	0.6206
Residual	3603.88	3	1201.29		
Cor. Total	1.11E+05	8			

Std. Dev.	34.66	R-Squared	0.9674	
Mean	1166.07	Adj. R-Squared	0.9131	
C.V. %	2.97	Pred. R-Squared	0.6536	
PRESS	38306.59	Adeq. Precision	9.986	

Table E. 2. Dimensional stability quadratic model of microwave/NaOH and microwave alone pretreated 1.6 mm screen size hammer mill oat hull pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	85.05954	5	17.01191	1.698484	0.3516
A- Alkali conc.	38.4054	1	38.4054	3.834429	0.1451
B- MW heating time	30.6456	1	30.6456	3.059684	0.1786
AB	1.8225	1	1.8225	0.18196	0.6985
A^2	13.65902	1	13.65902	1.363729	0.3273
B^2	0.527022	1	0.527022	0.052618	0.8333
Residual	30.04781	3	10.01594		
Cor. Total	115.1074	8			

Std. Dev.	3.164797	R-Squared	0.738958	
Mean	2.762222	Adj. R-Squared	0.303889	
C.V. %	114.5743	Pred. R-Squared	-1.78907	
PRESS	321.0427	Adeq. Precision	3.707365	

Table E. 3. Tensile strength quadratic model of microwave/NaOH and microwave alone pretreated 1.6 mm screen size hammer mill oat hull pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	8.11	5	1.62	8.47	0.0543
A- Alkali conc.	6.06	1	6.06	31.67	0.0111
B- MW heating time	0.9	1	0.9	4.69	0.119
AB	1.01	1	1.01	5.28	0.1052
A^2	0.13	1	0.13	0.66	0.4754
B^2	0.014	1	0.014	0.073	0.8051
Residual	0.57	3	0.19		
Cor. Total	8.68	8			

Std. Dev.	0.44	R-Squared	0.9339	
Mean	1.25	Adj. R-Squared	0.8237	
C.V. %	35.03	Pred. R-Squared	0.2529	
PRESS	6.49	Adeq. Precision	8.441	

Table E. 4. Pellet density quadratic model of microwave/KOH and microwave alone pretreated 1.6 mm screen size hammer mill oat hull pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	78872.81	5	15774.56	51.45	0.0042
A- Alkali conc.	70323.53	1	70323.53	229.35	0.0006
B- MW heating time	5851.88	1	5851.88	19.09	0.0222
AB	1071.91	1	1071.91	3.5	0.1583
A^2	1517.45	1	1517.45	4.95	0.1126
B^2	108.04	1	108.04	0.35	0.5946
Residual	919.86	3	306.62		
Cor. Total	79792.68	8			

Std. Dev.	17.51	R-Squared	0.9885	
Mean	1133.24	Adj. R-Squared	0.9693	
C.V. %	1.55	Pred. R-Squared	0.8677	
PRESS	10552.59	Adeq. Precision	19.513	

Table E. 5. Dimensional stability quadratic model of microwave/KOH and microwave alone pretreated 1.6 mm screen size hammer mill oat hull pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	74.47414	5	14.89483	2.346896	0.2569
A- Alkali conc.	36.40807	1	36.40807	5.73662	0.0963
B- MW heating time	24.12015	1	24.12015	3.80048	0.1463
AB	0.970225	1	0.970225	0.152873	0.7219
A^2	11.45609	1	11.45609	1.805073	0.2717
B^2	1.519606	1	1.519606	0.239436	0.6582
Residual	19.03982	3	6.346606		
Cor. Total	93.51396	8			

Std. Dev.	2.519247	R-Squared	0.796396	
Mean	2.902222	Adj. R-Squared	0.457056	
C.V. %	86.80408	Pred. R-Squared	-0.91469	
PRESS	179.0502	Adeq. Precision	4.344606	

Table E. 6. Tensile strength quadratic model of microwave/KOH and microwave alone pretreated 1.6 mm screen size hammer mill oat hull pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	1.33	5	0.27	8.7	0.0525
A- Alkali conc.	0.97	1	0.97	31.61	0.0111
B- MW heating time	0.21	1	0.21	6.83	0.0795
AB	0.1	1	0.1	3.34	0.1649
A^2	0.042	1	0.042	1.37	0.3259
B^2	9.80E-03	1	9.80E-03	0.32	0.6111
Residual	0.092	3	0.031		
Cor. Total	1.42	8			

Std. Dev.	0.17	R-Squared	0.9355	
Mean	0.61	Adj. R-Squared	0.8279	
C.V. %	28.69	Pred. R-Squared	0.4054	
PRESS	0.85	Adeq. Precision	8.539	

Table E. 7. Pellet density quadratic model of microwave/NaOH and microwave alone pretreated 3.2 mm screen size hammer mill oat hull pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	84846.05	5	16969.21	10.38	0.0413
A- Alkali conc.	77989.68	1	77989.68	47.69	0.0062
B- MW heating time	1330.57	1	1330.57	0.81	0.4335
AB	300.5	1	300.5	0.18	0.6971
A^2	5106.56	1	5106.56	3.12	0.1754
B^2	118.73	1	118.73	0.073	0.8051
Residual	4906.15	3	1635.38		
Cor. Total	89752.2	8			

Std. Dev.	40.44	R-Squared	0.9453	
Mean	1174.26	Adj. R-Squared	0.8542	
C.V. %	3.44	Pred. R-Squared	0.3578	
PRESS	57639.09	Adeq. Precision	7.808	

Table E. 8. Dimensional stability quadratic model of microwave/NaOH and microwave alone pretreated 3.2 mm screen size hammer mill oat hull pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	157.29	5	31.46	95.19	0.0017
A- Alkali conc.	16.5	1	16.5	49.93	0.0058
B- MW heating time	128.53	1	128.53	388.91	0.0003
AB	6.03	1	6.03	18.24	0.0236
A^2	4.18	1	4.18	12.64	0.038
B^2	2.06	1	2.06	6.23	0.0879
Residual	0.99	3	0.33		
Cor. Total	158.28	8			

Std. Dev.	0.57	R-Squared	0.9937
Mean	3.2	Adj. R-Squared	0.9833
C.V. %	17.98	Pred. R-Squared	0.9283
PRESS	11.35	Adeq. Precision	28.948

Table E. 9. Tensile strength quadratic model of microwave/NaOH and microwave alone pretreated 3.2 mm screen size hammer mill oat hull pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	5.75	5	1.15	19.34	0.0172
A- Alkali conc.	4.49	1	4.49	75.49	0.0032
B- MW heating time	0.81	1	0.81	13.69	0.0343
AB	0.32	1	0.32	5.37	0.1034
A^2	0.12	1	0.12	2.07	0.2455
B^2	5.34E-03	1	5.34E-03	0.09	0.784
Residual	0.18	3	0.059		
Cor. Total	5.93	8			

Std. Dev.	0.24	R-Squared	0.9699
Mean	1.27	Adj. R-Squared	0.9198
C.V. %	19.19	Pred. R-Squared	0.7304
PRESS	1.6	Adeq. Precision	12.388

Table E. 10. Pellet density quadratic model of microwave/KOH and microwave alone pretreated 3.2 mm screen size hammer mill oat hull pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	50959.73	5	10191.95	7.04	0.0695
A- Alkali conc.	48812.63	1	48812.63	33.74	0.0102
B- MW heating time	1338.03	1	1338.03	0.92	0.4072
AB	120.56	1	120.56	0.083	0.7916
A^2	116.84	1	116.84	0.081	0.7948
B^2	571.67	1	571.67	0.4	0.5742
Residual	4340.4	3	1446.8		
Cor. Total	55300.13	8			

Std. Dev.	38.04	R-Squared	0.9215	
Mean	1131.06	Adj. R-Squared	0.7907	
C.V. %	3.36	Pred. R-Squared	0.1508	
PRESS	46961.76	Adeq. Precision	7.01	

Table E. 11. Dimensional stability quadratic model of microwave/KOH and microwave alone pretreated 3.2 mm screen size hammer mill oat hull pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	127.3751	5	25.47502	13.33947	0.0292
A- Alkali conc.	9.200817	1	9.200817	4.817817	0.1157
B- MW heating time	108.46	1	108.46	56.79284	0.0048
AB	7.3984	1	7.3984	3.874019	0.1437
A^2	1.662272	1	1.662272	0.870414	0.4197
B^2	0.653606	1	0.653606	0.342247	0.5996
Residual	5.729244	3	1.909748		
Cor. Total	133.1044	8			

Std. Dev.	1.381936	R-Squared	0.956957	
Mean	4.402222	Adj. R-Squared	0.885218	
C.V. %	31.39179	Pred. R-Squared	0.530442	
PRESS	62.50023	Adeq. Precision	9.94671	

Table E. 12. Tensile strength quadratic model of microwave/KOH and microwave alone pretreated 3.2 mm screen size hammer mill oat hull pellets.

Source	Sum of squares	df	Mean square	F value	P-value Prob. > F
Model	0.98	5	0.2	12.61	0.0315
A- Alkali conc.	0.86	1	0.86	55.43	0.005
B- MW heating time	0.11	1	0.11	7.23	0.0744
AB	1.00E-04	1	1.00E-04	6.46E-03	0.941
A^2	3.47E-03	1	3.47E-03	0.22	0.6682
B^2	2.69E-03	1	2.69E-03	0.17	0.705
Residual	0.046	3	0.015		
Cor. Total	1.02	8			

Std. Dev.	0.12	R-Squared	0.9546	
Mean	0.72	Adj. R-Squared	0.8789	
C.V. %	17.39	Pred. R-Squared	0.5556	
PRESS	0.45	Adeq. Precision	10.135	

F. The 3D Response Surface and the 2D Contour Plots of the Responses from Microwave-Assisted Alkali Pretreated Canola Straw Pellets.
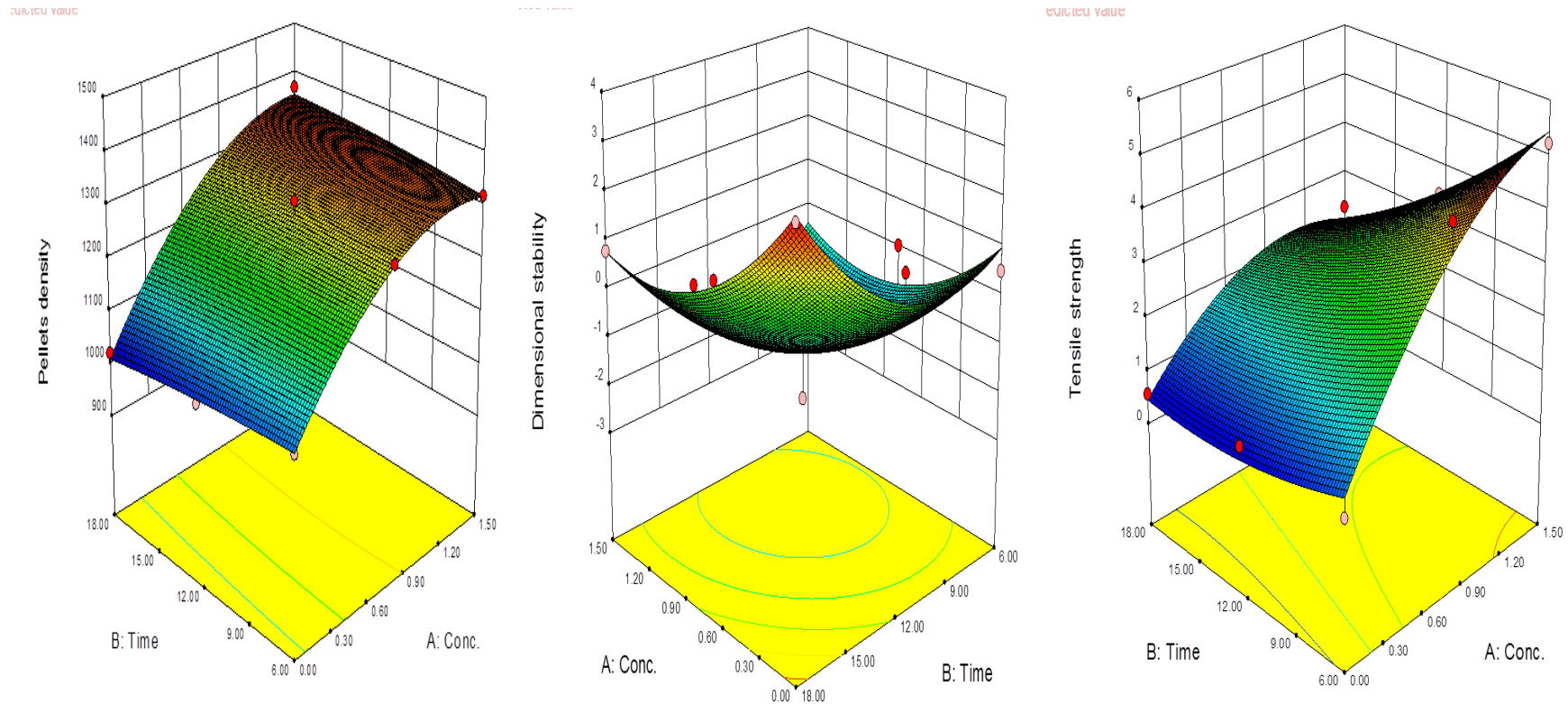
Figure F. 1. Response surface for the effects of alkali conc. (%) and MW heating time (min) on microwave-assisted alkali pretreated 1.6 mm Canola Straw pellets using NaOH.
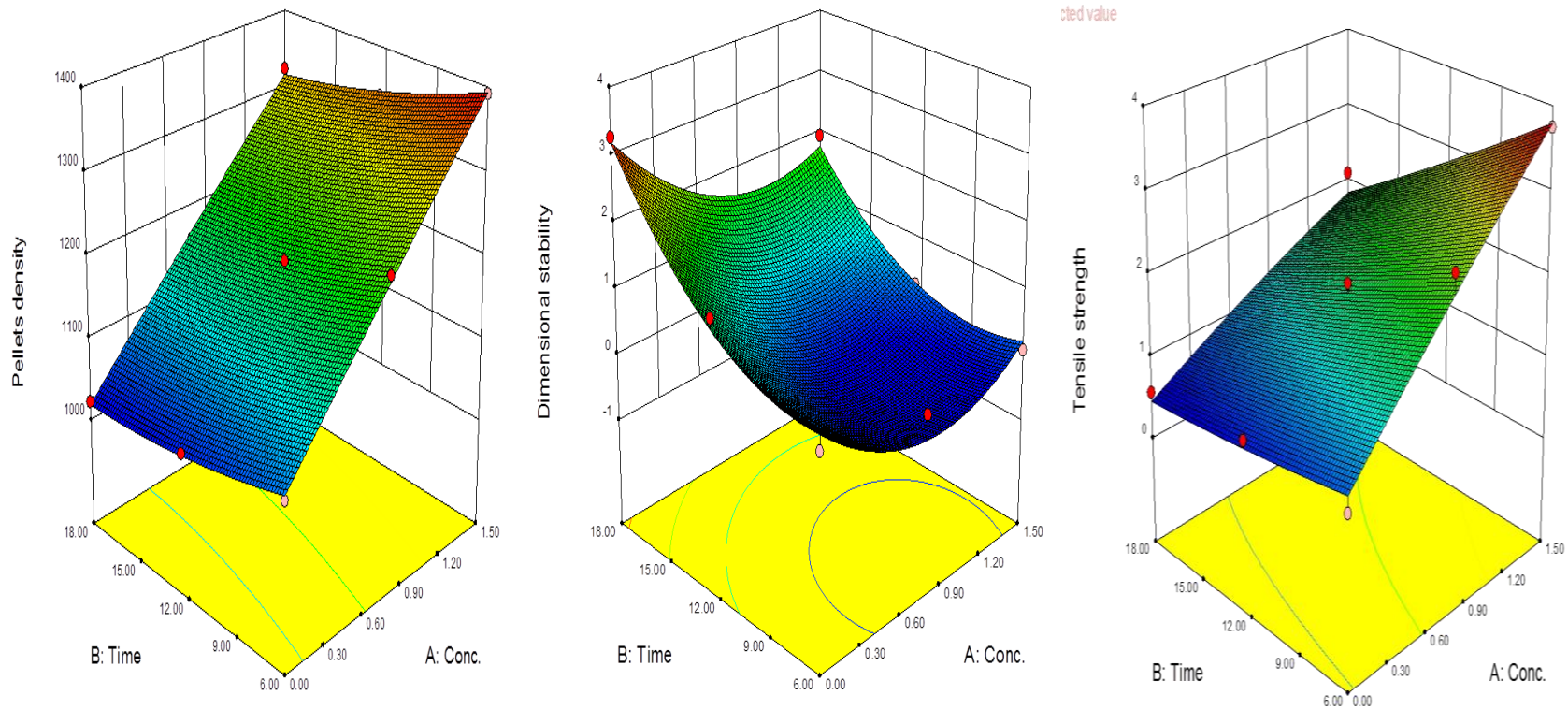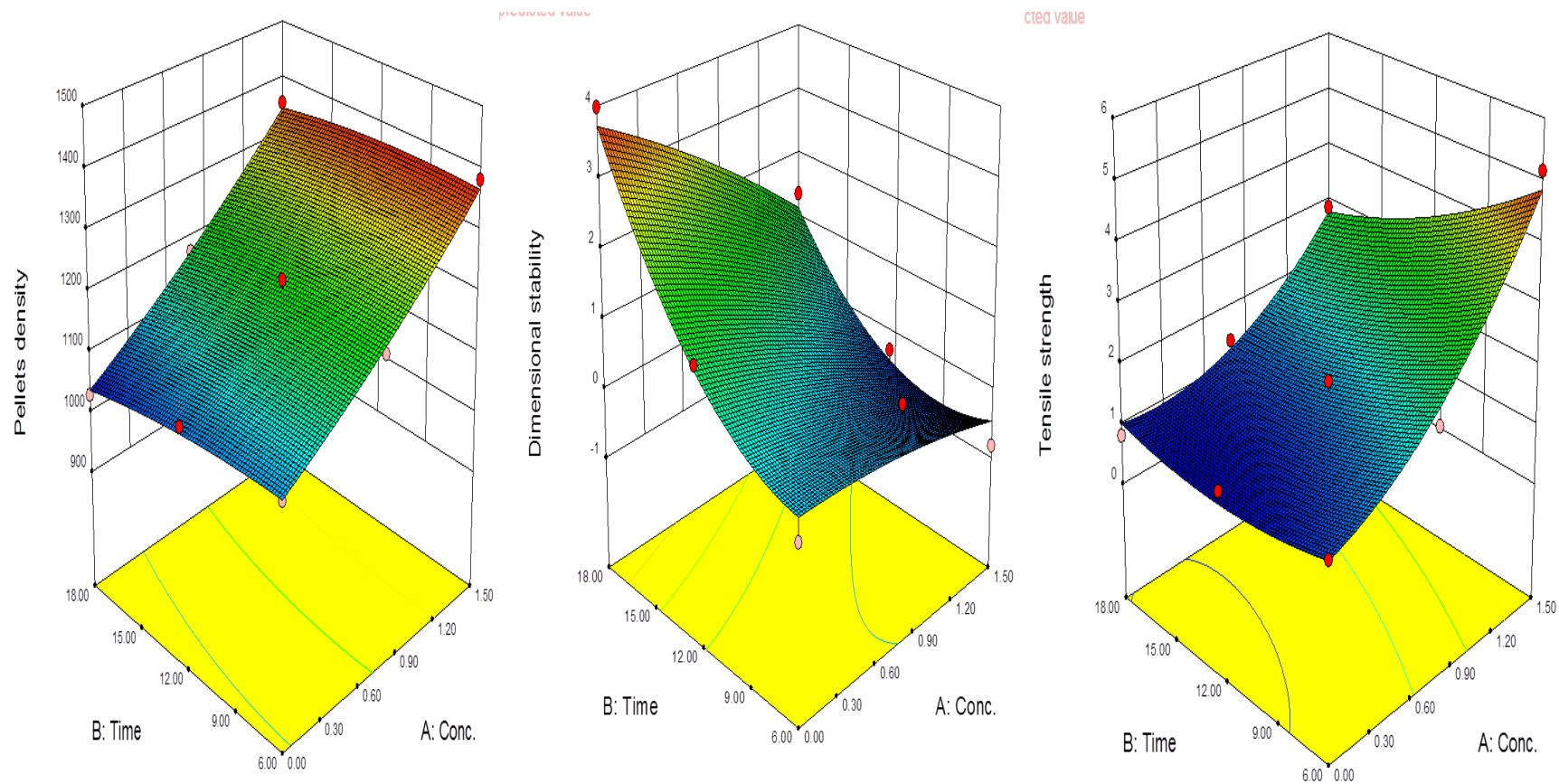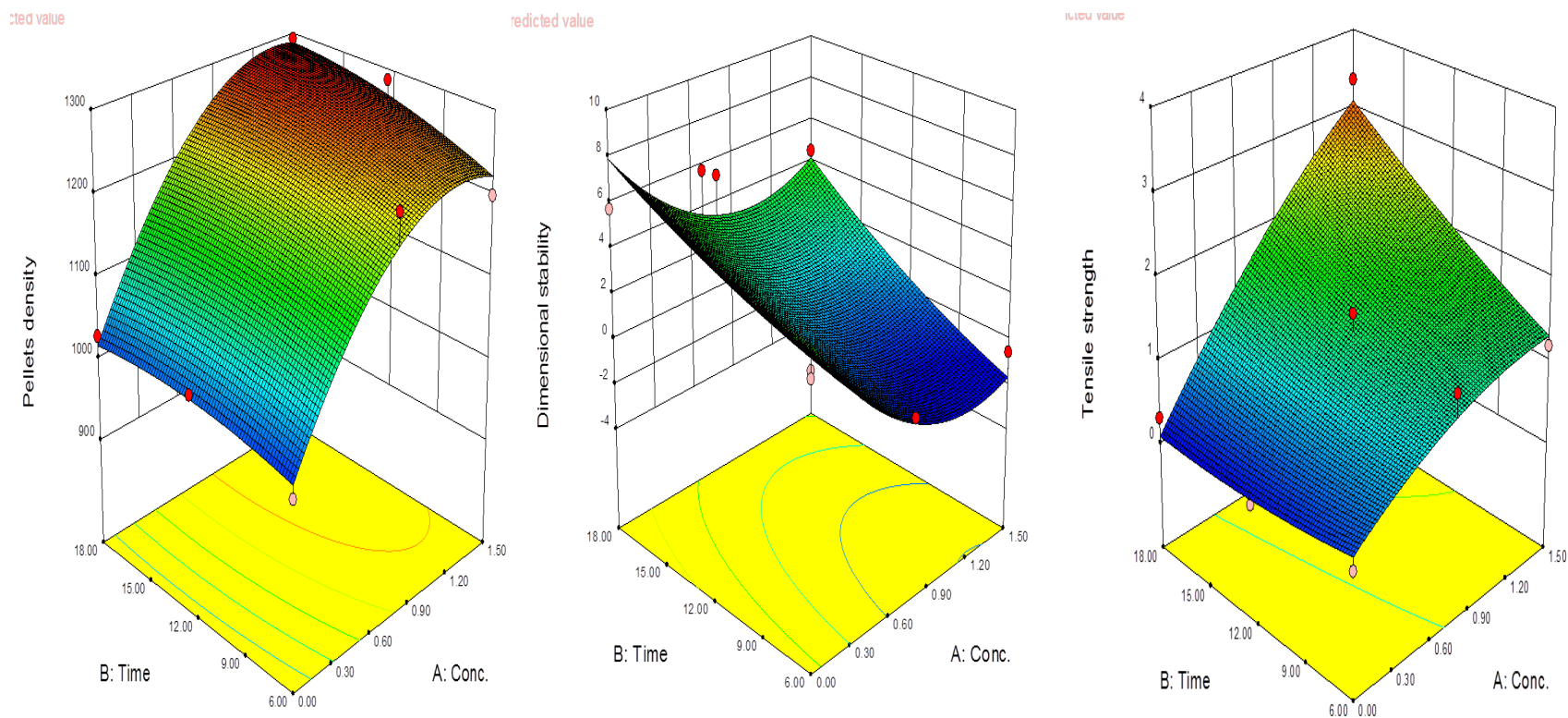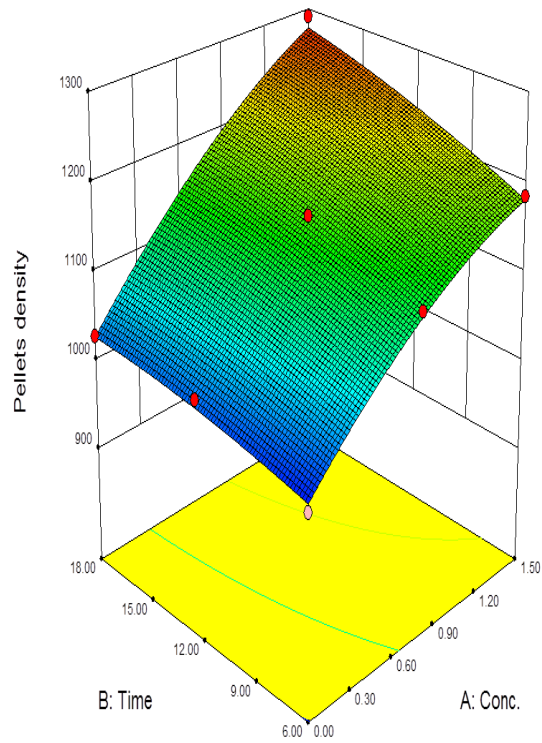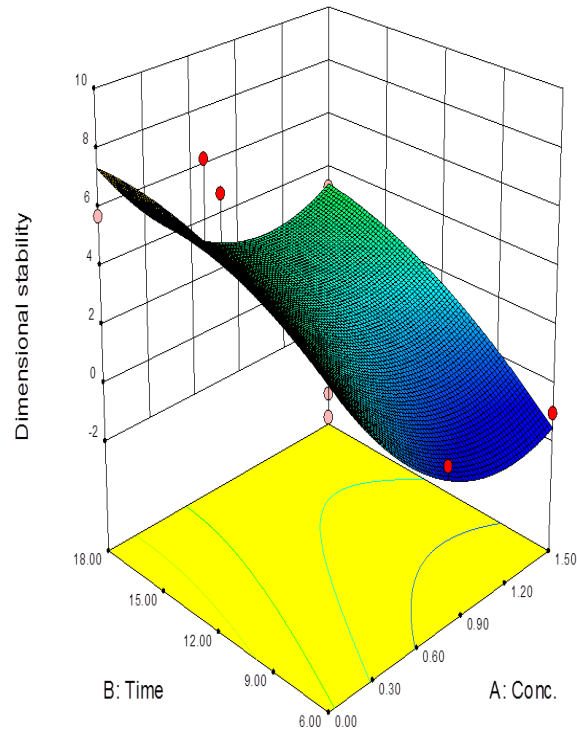
Figure F. 2. Response surface for the effects of alkali conc. (%) and MW heating time (min) on microwave-assisted alkali pretreated 1.6 mm Canola Straw pellets using KOH.
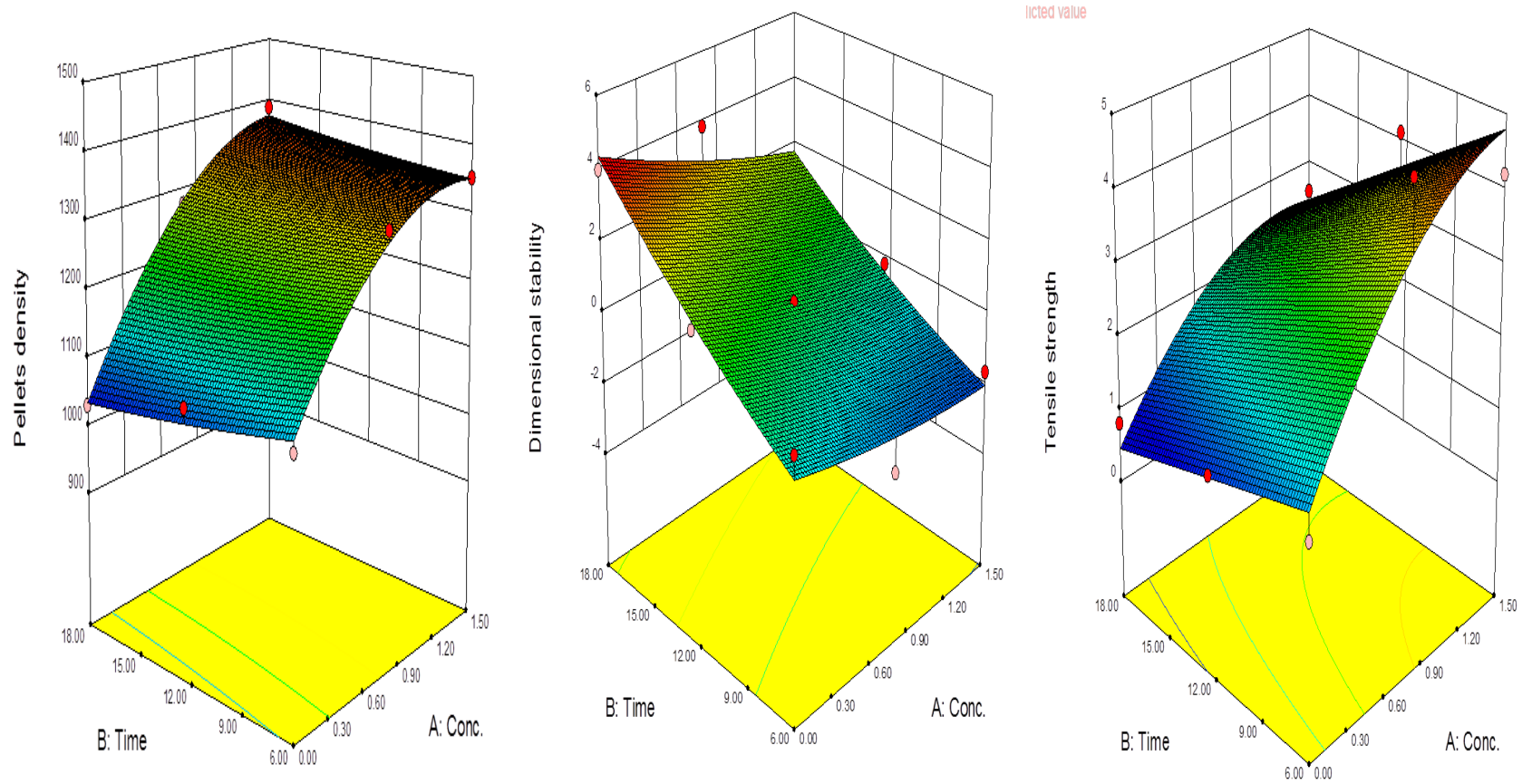
Figure F. 3. Response surface for the effects of alkali conc. (%) and MW heating time (min) on microwave-assisted alkali pretreated 3.2 mm Canola Straw pellets using NaOH.
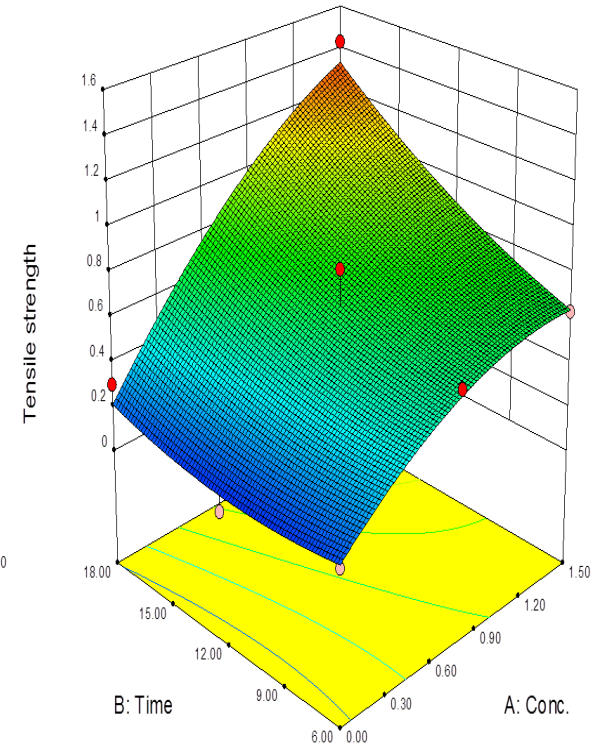
Figure F. 4. Response surface for the effects of alkali conc. (%) and MW heating time (min) on microwave-assisted alkali pretreated 3.2 mm Canola Straw pellets using KOH.

G. The 3D Response Surface and the 2D Contour Plots of the Responses from Microwave-Assisted Alkali Pretreated Oat Hull Pellets.
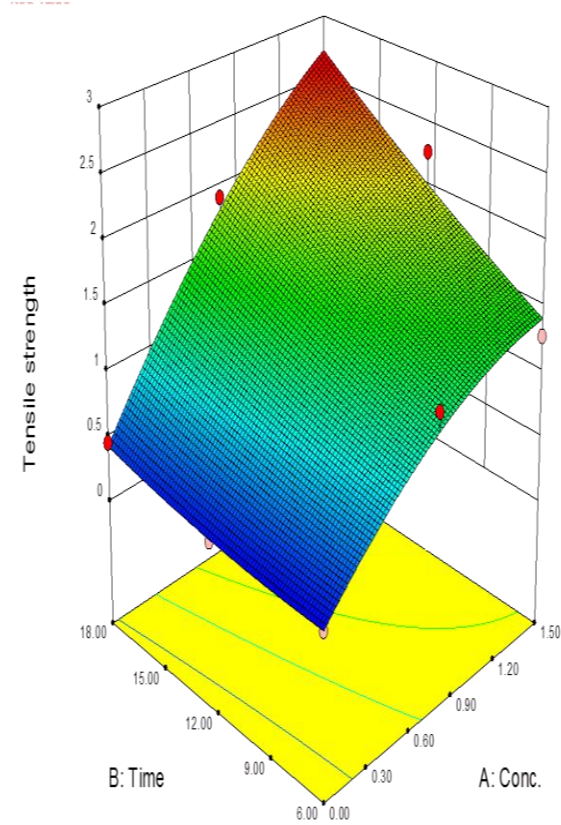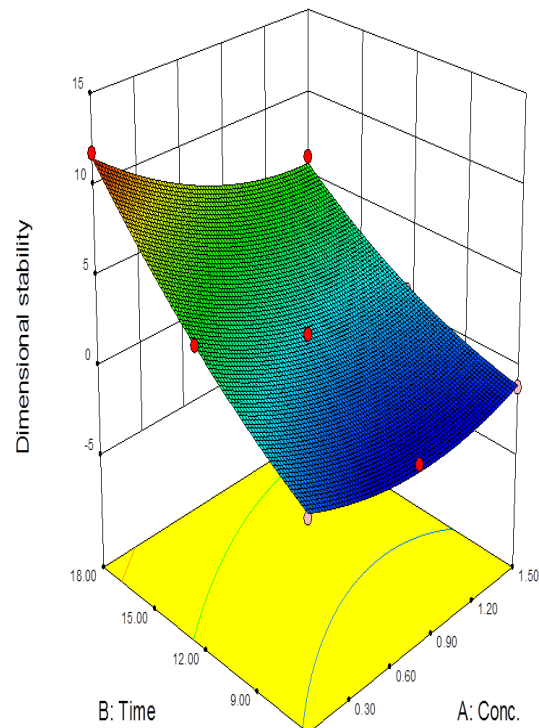

Figure G. 1. Response surface for the effects of alkali conc. (%) and MW heating time (min) on microwave-assisted alkali pretreated 1.6 mm oat hull pellets using NaOH.

Figure G. 2. Response surface for the effects of alkali conc. (%) and MW heating time (min) on microwave-assisted alkali pretreated 1.6 mm oat hull pellets using KOH.
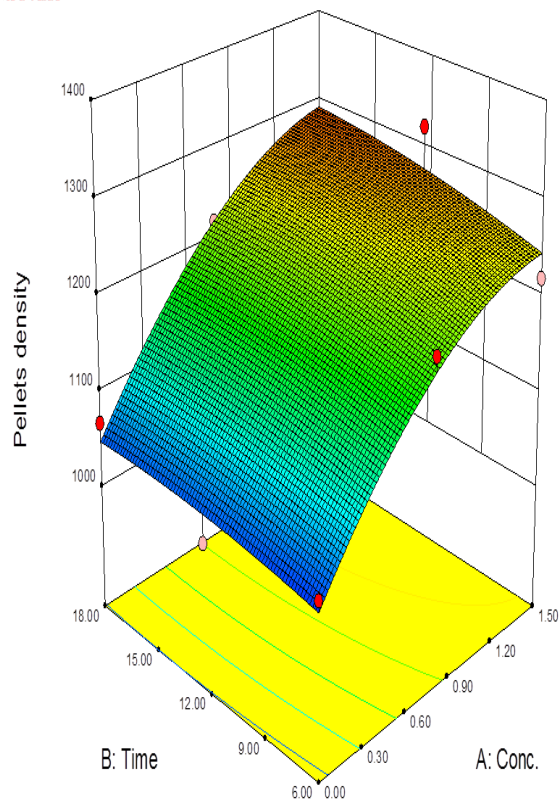
Figure G. 3. Response surface for the effects of alkali conc. (%) and MW heating time (min) on microwave-assisted alkali pretreated 3.2 mm oat hull pellets using NaOH.
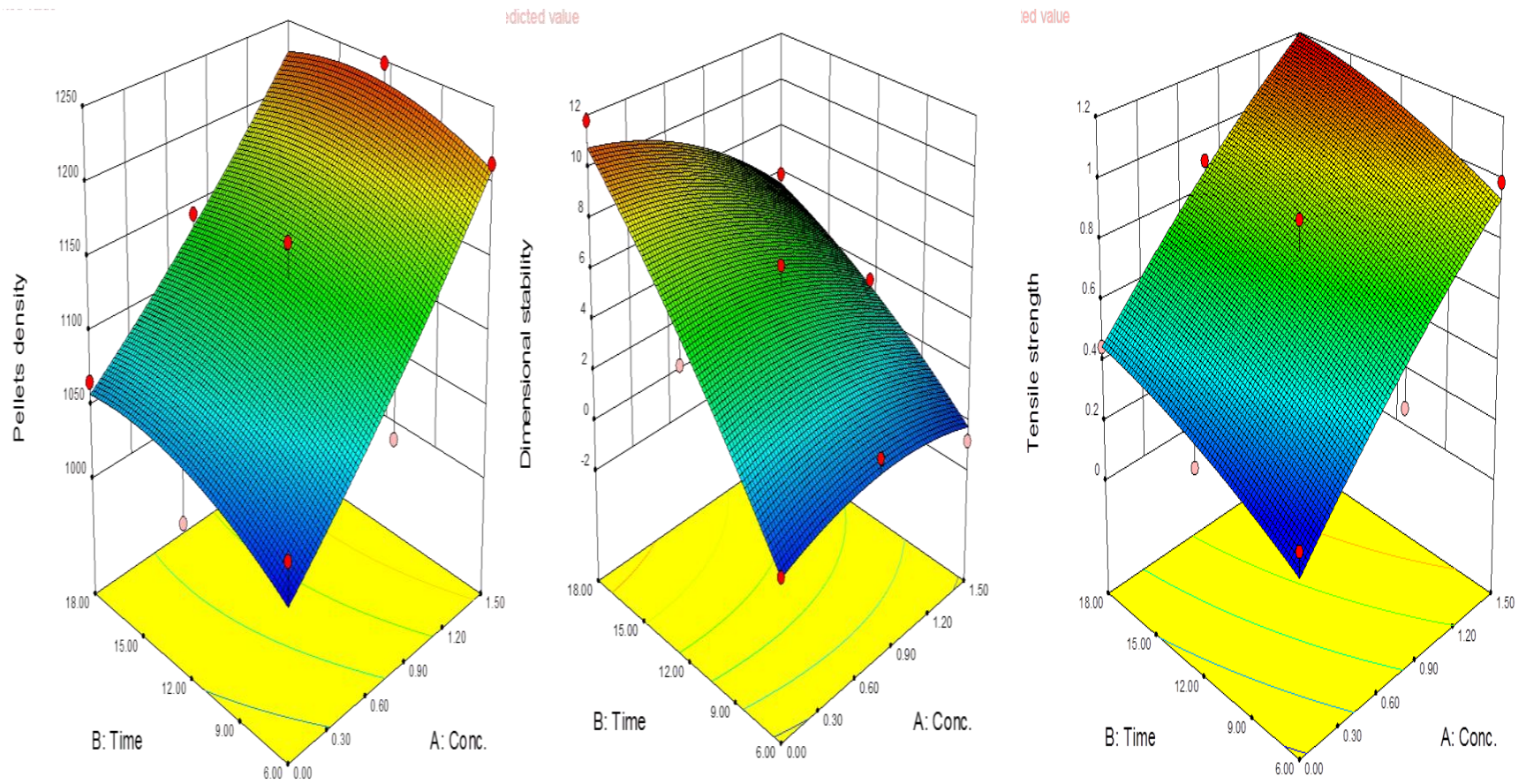
Figure G. 4. Response surface for the effects of alkali conc. (%) and MW heating time (min) on microwave-assisted alkali pretreated 3.2 mm oat hull pellets using KOH.

Appendix H

H. Regression Equations for Microwave-Assisted Alkali Pretreated Canola Straw and Oat Hull Pellets Physical Performance

Table H. 1. Regression equations for microwave-assisted alkali pretreated canola straw pellets physical performance.

Physical performance	Response surface model
Microwave/NaOH pretreated 1.6 mm canola straw pellets	
Tensile strength	$2.87 + 1.49A - 1.00B - 0.69AB - 0.89A^2 + 0.27B^2$
Dimensional stability	$1.24 - 0.99A + 0.90B - 0.30AB + 1.09A^2 - 1.35B^2$
Pellet density	$1285.47 + 148.76A - 5.30B + 23.90AB - 90.99A^2 - 6.12B^2$
Microwave/KOH pretreated 1.6 mm canola straw pellets	
Tensile strength	$1.78 + 1.07A - 0.60B - 0.37AB - 0.063A^2 + 0.037B^2$
Dimensional stability	$0.036 - 0.49A + 1.00B - 0.22AB + 0.81A^2 + 0.71B^2$
Pellet density	$1192.00 + 155.23A - 32.52B - 5.82AB - 2.62A^2 + 10.60B^2$
Microwave/NaOH pretreated 3.2 mm canola straw pellets	
Tensile strength	$3.03 + 1.31A - 0.86B - 0.31AB - 0.70A^2 - 0.005B^2$
Dimensional stability	$0.33 - 0.95A + 2.24B - 0.33AB + 0.21A^2 + 0.3B^2$
Pellet density	$1292.00 + 146.60A - 11.69B + 24.01AB - 79.99A^2 + 4.81B^2$
Microwave/KOH pretreated 3.2 mm canola straw pellets	
Tensile strength	$1.56 + 1.37A - 0.55B - 0.47AB + 0.64A^2 + 0.27B^2$
Dimensional stability	$0.50 - 0.80A + 1.29B - 0.48AB - 0.14A^2 + 0.79B^2$
Pellet density	$1206.91 + 146.13A - 18.51B + 8.17AB + 15.37A^2 - 11.28B^2$

A: alkali concentration (%); B: microwave heating time (min)

Table H. 2. Regression equations for microwave-assisted alkali pretreated oat hull pellets physical performance.

Physical performance	Response surface model
Microwave/NaOH pretreated 1.6 mm oat hull pellets	
Tensile strength	$1.36 + 1.01A + 0.39B + 0.50AB - 0.25A^2 + 0.083B^2$
Dimensional stability	$0.68 - 2.26A + 2.28B + 0.68AB + 2.61A^2 + 0.51B^2$
Pellet density	$1232.03 + 121.77A + 19.53B + 13.53AB - 85.51A^2 - 13.48B^2$
Microwave/KOH pretreated 1.6 mm oat hull pellets	
Tensile strength	$0.66 + 0.40A + 0.19B + 0.16AB - 0.14A^2 + 0.070B^2$
Dimensional stability	$1.89 - 2.46A + 2.00B + 0.49AB + 2.39A^2 - 0.87B^2$
Pellet density	$1156.50 + 108.26A + 31.23B + 16.37AB - 27.54A^2 - 7.35B^2$
Microwave/NaOH pretreated 3.2 mm oat hull pellets	
Tensile strength	$1.40 + 0.87A + 0.37B + 0.28AB - 0.25A^2 + 0.052B^2$
Dimensional stability	$1.56 - 1.66A + 4.63B - 1.23AB + 1.45A^2 + 1.01B^2$
Pellet density	$1213.09 + 114.01A + 14.89B + 8.67AB - 50.53A^2 - 7.70B^2$
Microwave/KOH pretreated 3.2 mm oat hull pellets	
Tensile strength	$0.77 + 0.38A + 0.14B - 0.005AB - 0.042A^2 - 0.037B^2$
Dimensional stability	$5.39 - 1.24A + 4.25B - 1.36AB - 0.91A^2 - 0.57B^2$
Pellet density	$1137.23 + 90.20A + 14.93B - 5.49AB + 7.64A^2 - 16.91B^2$

A: alkali concentration (%); B: microwave heating time (min)