

A Study on the Extraction Methods and Characterizations of *Typha* Leaf Fibers

by

Chun Hang Yu

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfilment of the requirements of the degree of

MASTER OF SCIENCE

Department of Biosystems Engineering

University of Manitoba

Winnipeg

Copyright © 2021 by Chun Hang Yu

Abstract

Typha leaf fibers are potentially useful resources in the fiber engineering field. Chemical extraction was a common method to extract fibers from the *Typha* leaves. However, depending on the extraction protocol, the fibers obtained might be less regular with a wider range of diameters. The leaves might react with the alkaline solution to a varying extent and fiber products with inconsistent quality might be formed. This research investigated the morphology of fibers from manual and chemical extraction. The presence of calcium oxalate plates on a number of fibers indicated that lacunae fiber (also called fiber cable in literature) could be one type of extracted fiber in the chemical extraction, but whether it was the only type of extracted fiber was not clear. Lacunae fibers could be manually extracted from the leaves by removing the surrounding leaf materials. A manual extraction method was designed and explained in this research. The manually extracted lacunae fibers had the surface morphology of nodes and calcium oxalate plates. The node might be the remainder of the diaphragm in the leaf structure. Chemical treatment using potassium hydroxide solution for the manually extracted lacunae fibers was able to reduce the mean and variance of fiber diameters. The post-extraction process was important for both manual and chemical extraction as it could affect the quality of the fibers and should be further investigated. This research found that extracting the lamina, leaf sheath, and leaf sheath without the dorsal and ventral surface chemically produced fibers with different colors.

Acknowledgements

I would like to thank my research advisor Dr. Mashiur Rahman for his guidance on this research, Dr. Jason Morrison for his advice on part of the statistical analyses, and Manitoba Institute for Materials for the characterization of samples.

Table of contents

Abstract	i
Acknowledgements	ii
Table of contents	iii
List of Tables	vi
List of Figures	viii
CHAPTER 1. Introduction.....	1
1.1 Cattails and their interaction with wildlife	1
1.2 Weed problem	1
1.3 Scientific application - phytoremediation	3
1.4 Other scientific applications - <i>Typha</i> fibers	4
1.5 Objectives.....	6
CHAPTER 2. Literature review.....	7
2.1 Botanical studies on the <i>Typha</i> leaf structure.....	7
2.2 Definition of a fiber	8
2.3 Chemical extraction of <i>Typha</i> fibers	8
2.4 <i>Typha</i> fibers diameter.....	9
CHAPTER 3. Materials and methods	11
3.1 Materials.....	11
3.2 Chemicals	11
3.3 Chemical extraction and chemical treatment	11
3.4 Diameter measurement.....	12
3.5 Environmental scanning electron microscopy (ESEM) imaging.....	12
3.6 Energy-dispersive X-ray spectroscopy (EDS) analysis	13

3.7 Statistical analyses.....	13
CHAPTER 4. Manual extraction of lacunae fibers	14
4.1 Manual extraction method.....	14
4.2 Characterization of the fibers	28
4.2.1 Morphology	28
4.2.2 Diameter	36
4.2.3 Properties of the calcium oxalate plates	37
4.3 Botanical study on the extraction.....	39
CHAPTER 5. Treatment of the manually extracted lacunae fibers.....	41
5.1 Treatment with an alkaline chemical at room temperature	41
5.2 Characterization of the fibers with alkaline treatment at room temperature.....	42
5.2.1 Morphology	43
5.2.2 Diameter	49
5.3 Treatment with an alkaline chemical at higher temperature	49
5.4 Characterization of the fibers with alkaline treatment at higher temperature	49
5.4.1 Morphology	50
5.4.2 Diameter	54
CHAPTER 6. Chemically extracted <i>Typha</i> leaf fibers	55
6.1 Chemically extracted fibers.....	55
6.2. Characterization of the chemically extracted fibers	56
6.2.1 Morphology	56
6.2.2 Calcium oxalate plates and pits	68
6.2.3 Color	71
CHAPTER 7. Comparison between different types of extracted fibers and the discussion of <i>Typha</i> leaf fiber extraction	75

7.1 Comparison between different types of extracted fibers.....	75
7.1.1 Mean and variance of diameters.....	75
7.1.2 Surface morphology	77
7.2 Discussion	78
7.2.1 The diameter problem.....	78
7.2.2 The fibers obtained from the chemical extraction.....	79
7.2.3 The target fiber structure	81
7.2.4 The surface morphology problems	82
7.2.5 The significance of the post-extraction process	83
7.2.6 Fiber extraction of different parts of a leaf.....	84
CHAPTER 8. Conclusions.....	86
References.....	89
Appendix I – Data of the diameter measurement	93
Appendix II – EDS results for labels in Figure 4.2.3.1.....	96
Appendix III – EDS results for labels in Figure 6.2.2.1	109
Appendix IV – Statistical analysis.....	116

List of Tables

<i>Table 6.2.2.1. EDS results on a chemically extracted fiber.....</i>	<i>70</i>
<i>Table 7.1.1.1. Diameter measurement results and statistical data.....</i>	<i>75</i>
<i>Table 7.1.1.2. F-test results</i>	<i>75</i>
<i>Table 7.1.1.3. Welch two sample t-test results.....</i>	<i>76</i>
<i>Table A1.1. The diameter data for the manually extracted lacunae fibers (not including the nodes).....</i>	<i>93</i>
<i>Table A1.2. The diameter data for the chemically treated (at room temperature) manually extracted lacunae fibers (not including the nodes).....</i>	<i>94</i>
<i>Table A1.3. The diameter data for the chemically treated (at 70 – 80°C) manually extracted lacunae fibers (not including the nodes).....</i>	<i>95</i>
<i>Table A2.1. EDS results for label 1 (the plate) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.</i>	<i>96</i>
<i>Table A2.2. EDS results for label 2 (the plate) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.</i>	<i>97</i>
<i>Table A2.3. EDS results for label 3 (the plate) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.</i>	<i>98</i>
<i>Table A2.4. EDS results for label 4 (the plate) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.</i>	<i>99</i>
<i>Table A2.5. EDS results for label 5 (the plate) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.</i>	<i>100</i>
<i>Table A2.6. EDS results for label 6 (the plate) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.</i>	<i>101</i>
<i>Table A2.7. EDS results for label 7 (the plate) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.</i>	<i>102</i>
<i>Table A2.8. EDS results for label 8 (the non-plate place) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.</i>	<i>103</i>
<i>Table A2.9. EDS results for label 9 (the non-plate place) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.</i>	<i>104</i>

<i>Table A2.10. EDS results for label 10 (the non-plate place) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.</i>	<i>105</i>
<i>Table A2.11. EDS results for label 11 (the non-plate place) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.</i>	<i>106</i>
<i>Table A2.12. EDS results for label 12 (the non-plate place) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.</i>	<i>107</i>
<i>Table A2.13. EDS results for label 13 (the non-plate place) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.</i>	<i>108</i>
<i>Table A3.1. EDS results for label 1 (the pit) in Figure 6.2.2.1. The letter “K” was from the raw data and it indicated the energy-level shell.</i>	<i>109</i>
<i>Table A3.2. EDS results for label 2 (the calcium oxalate plate) in Figure 6.2.2.1. The letter “K” was from the raw data and it indicated the energy-level shell.</i>	<i>110</i>
<i>Table A3.3. EDS results for label 3 (the non-plate place) in Figure 6.2.2.1. The letter “K” was from the raw data and it indicated the energy-level shell.</i>	<i>111</i>
<i>Table A3.4. EDS results for label 4 (the non-plate place) in Figure 6.2.2.1. The letter “K” was from the raw data and it indicated the energy-level shell.</i>	<i>112</i>
<i>Table A3.5. EDS results for label 5 (the pit) in Figure 6.2.2.1. The letter “K” was from the raw data and it indicated the energy-level shell.</i>	<i>113</i>
<i>Table A3.6. EDS results for label 6 (the pit) in Figure 6.2.2.1. The letter “K” was from the raw data and it indicated the energy-level shell.</i>	<i>114</i>
<i>Table A3.7. EDS results for label 7 (the remainder of the calcium oxalate plate) in Figure 6.2.2.1. The letter “K” was from the raw data and it indicated the energy-level shell.....</i>	<i>115</i>

List of Figures

<i>Figure 4.1.1. Cross-section of a Typha leaf.</i>	15
<i>Figure 4.1.2. Before or after compressing the Typha leaf, the thinner parts of the leaf at both sides could be removed, which was indicated by the green lines (into the paper). Compress the Typha leaf as shown by the purple arrows and cut the dorsal surface according to the red dashed line (into the paper).</i>	15
<i>Figure 4.1.3. Top view of a Typha leaf. Cut the dorsal surface longitudinally. The yellow arrow indicated the cutting direction.</i>	16
<i>Figure 4.1.4. An ESEM image of the cross-section of a Typha leaf. Before or after compressing the Typha leaf, the thinner parts of the leaf at both sides could be removed, which was indicated by the green lines (into the paper). The red dashed line indicated the starting position to cut the dorsal surface (into the paper). The red dashed line should be straighter after the compression.</i>	17
<i>Figure 4.1.5. The top view of a Typha leaf with the dorsal surface removed.</i>	18
<i>Figure 4.1.6. The top view of a Typha leaf with the dorsal surface removed. Brown arrowheads on the top indicated the partitions. Black arrowheads at the right of the red dashed line indicated the diaphragms. Purple arrowheads at the bottom indicated the lacunae fibers. A column could be separated by cutting along the red dashed lines.</i>	19
<i>Figure 4.1.7. An ESEM image of the top view of a Typha leaf with the dorsal surface removed. The blue arrow indicated the non-diaphragm aerenchyma. The brown arrowheads on the top indicated the partitions. The area with orange dashed sides indicated the diaphragm. The purple arrowheads at the bottom indicated the lacunae fibers (the target fibers of the manual extraction method). A column could be separated by cutting along the red dashed lines. The yellow scale bar indicated 500 μm.</i>	20
<i>Figure 4.1.8. The image on the left was a shorter section of a removed column. It was the side view. The black arrowhead indicated the ventral surface. The image on the right was the scaled up image of the image on the left. The ventral surface could be separated by cutting along the red dashed line.</i>	21
<i>Figure 4.1.9. The substance on the left was the column, which had the ventral surface removed. The purple arrowheads indicated the place for pinching. The blue arrow indicated the longitudinal direction for pulling after the pinching. The substance on the right was the ventral surface.</i>	24

<i>Figure 4.1.10. The column on the left contained partition materials, which caused a difficult extraction process; the column on the right did not contain or contained fewer partition materials and therefore an easier extraction process resulted.</i>	<i>25</i>
<i>Figure 4.1.11. An ESEM image of a column with part of the non-diaphragm aerenchyma and diaphragms removed. The purple arrowheads indicated the approximate place for pinching.</i>	<i>26</i>
<i>Figure 4.1.12. A bunch of extracted fibers, which were not only obtained from a single column.</i>	<i>27</i>
<i>Figure 4.2.1.1. An ESEM image of a lacunae fiber obtained from the manual extraction method. Nodes (indicated by the yellow arrows) could be observed. There were calcium oxalate plates on this fiber, but they were difficult to be observed in this image.</i>	<i>29</i>
<i>Figure 4.2.1.2. An ESEM image of a closer view of a manually extracted lacunae fiber. Calcium oxalate plates (an example was indicated by the yellow arrow) could be observed.</i>	<i>30</i>
<i>Figure 4.2.1.3. An ESEM image of a closer view of a manually extracted lacunae fiber. A node (indicated by the area with a red dashed side) and calcium oxalate plates could be observed. ..</i>	<i>31</i>
<i>Figure 4.2.1.4. An ESEM image of a larger magnification of Figure 4.1.7, which showed a lacunae fiber anchored (indicated by the area with a yellow dotted side) in a diaphragm with a partition next to the diaphragm (indicated by the yellow arrow).</i>	<i>32</i>
<i>Figure 4.2.1.5. An ESEM image of the structure of a Typha leaf. The cross-section of the partition was between the yellow dashed lines. The non-diaphragm aerenchymae were at both sides of the partition. The yellow scale bar indicated 100 μm.</i>	<i>33</i>
<i>Figure 4.2.1.6. An ESEM image showing an enclosed Typha leaf lacunae fiber (indicated by the area with yellow dashed sides). The cross-section of it was shown.</i>	<i>34</i>
<i>Figure 4.2.1.7. An ESEM image of a manually extracted Typha leaf lacunae fiber. The cross-section of it was shown.</i>	<i>35</i>
<i>Figure 4.2.3.1. A backscattered electron image of a manually extracted lacunae fiber. Label 1 – 13 were the detection focuses (labels with different colors) of the energy-dispersive X-ray spectroscopy. Label 1 – 7 focused on the plates and label 8 – 13 focused on the non-plate places.</i>	<i>37</i>
<i>Figure 4.2.3.2. EDS results for label 1 (the plate) in Figure 4.2.3.1</i>	<i>38</i>
<i>Figure 4.2.3.3. EDS results for label 8 (the non-plate place) in Figure 4.2.3.1</i>	<i>38</i>
<i>Figure 5.2.1.1. An ESEM image of a lacunae fiber obtained from the manual extraction method and treated with 5 wt% KOH solution for 2 hours at room temperature. Nodes and calcium oxalate plates could be observed.</i>	<i>44</i>

<i>Figure 5.2.1.2. An ESEM image of a closer view of a chemically treated (at room temperature) manually extracted lacunae fiber. A node and calcium oxalate plates could be observed.</i>	<i>45</i>
<i>Figure 5.2.1.3. An ESEM image of a closer view of a chemically treated (at room temperature) manually extracted lacunae fiber with a higher magnification. A node and calcium oxalate plates could be observed.</i>	<i>46</i>
<i>Figure 5.2.1.4. A backscattered electron image of a chemically treated (at room temperature) manually extracted lacunae fiber. Calcium oxalate plates could be observed.....</i>	<i>47</i>
<i>Figure 5.2.1.5. A backscattered electron image of a closer view of a chemically treated (at room temperature) manually extracted lacunae fiber. Calcium oxalate plates could be observed.....</i>	<i>48</i>
<i>Figure 5.4.1.1. An ESEM image of a lacunae fiber obtained from the manual extraction method and treated with 5 wt% KOH solution for 2 hours at 70 – 80°C. Nodes and calcium oxalate plates could be observed.</i>	<i>51</i>
<i>Figure 5.4.1.2. An ESEM image of a closer view of a chemically treated (at 70 – 80°C) manually extracted lacunae fiber. Calcium oxalate plates could be observed.</i>	<i>52</i>
<i>Figure 5.4.1.3. A backscattered electron image of a chemically treated (at 70 – 80°C) manually extracted lacunae fiber. Calcium oxalate plates could be observed.</i>	<i>53</i>
<i>Figure 6.2.1.1. An ESEM image showing different sizes of products from the chemical extraction. The oval shapes below should not be related to the cattail fibers. This image has shown a fiber with an observably larger diameter than the other thinner fibers (indicated by the yellow arrow).</i>	<i>56</i>
<i>Figure 6.2.1.2. A backscattered electron image capturing the same place as Figure 6.2.1.1. Calcium oxalate plates appeared to have a whiter color in this mode. All (or most) of the fibers had calcium oxalate plates on their surfaces in this image.</i>	<i>57</i>
<i>Figure 6.2.1.3. This ESEM image had a larger magnification to zoom in on the fiber with a relatively larger diameter in Figure 6.2.1.1. It could be observed that there were potentially some unremoved aerenchyma (an example was indicated by the area with yellow dashed sides). Apart from that, for the fiber with calcium oxalate plates (indicated by the yellow arrow), if only a column of calcium oxalate plates was focused, four pits could be observed (indicated by the four red arrowheads). They were deduced to be the surface morphology of the lacunae fiber after the calcium oxalate plates were removed from these positions.</i>	<i>58</i>
<i>Figure 6.2.1.4. A backscattered electron image capturing the same place as Figure 6.2.1.3. Calcium oxalate plates appeared to have a whiter color in this mode. The column of calcium oxalate plates and places which had the calcium oxalate plates removed were indicated by the red lines and arrowheads.....</i>	<i>59</i>

Figure 6.2.1.5. An ESEM image showing different sizes of products from the chemical extraction. The oval shapes below should not be related to the cattail fibers. This image captured fibers with a large variation in diameter. For example, the two yellow arrowheads indicated the two fibers with an observably larger and smaller diameter. There was a fiber or fiber bundle (depends on the definition of a fiber bundle) which turned around at a position and formed a loop (indicated by the yellow arrow). Its thinner structural fibers had been separated and the ESEM image with a higher magnification on this part could be referred to Figure 6.2.1.7..... 60

Figure 6.2.1.6. A backscattered electron image capturing the same place as Figure 6.2.1.5. Some fibers had calcium oxalate plates on their surfaces in this image. 61

Figure 6.2.1.7. This ESEM image had a larger magnification to zoom in on the loop position in Figure 6.2.1.5. It could be observed that the structural fibers were separated at this loop position with both ends being bundles. The reason of having a loop was not the focus here, but the structural fibers with smaller diameters found in this loop showing its structural dissociation were..... 62

Figure 6.2.1.8. A backscattered electron image capturing the same place as Figure 6.2.1.7. The whiter substances should be the calcium oxalate materials. There was calcium oxalate debris on the fiber loop. An example was indicated by the red arrowhead..... 63

Figure 6.2.1.9. A backscattered electron image capturing a twisted fiber (or fiber bundle) near the loop in Figure 6.2.1.7 and 6.2.1.8. The reason of having a bend was not the focus here, but the structural fibers with smaller diameters found in this bend showing its structural dissociation were..... 64

Figure 6.2.1.10. A backscattered electron image with a larger magnification zoomed in on Figure 6.2.1.9. Calcium oxalate debris could be observed on the fiber (or fiber bundle). An example was indicated by the red arrowhead..... 65

Figure 6.2.1.11. A backscattered electron image showing the fibers with different morphology. (a). The division of the smaller diameter structural fibers became clear. They appeared to be a less ordered bundle than (d). (b). The less structurally dissociated fiber, which had many calcium oxalate plates on the surface. (c). The section of the fiber where the calcium oxalate plate and pit could be found. (d). The section of the fiber where the surface morphology of pit was not obvious or was different from (c), while large calcium oxalate plates could not be found on this section of fiber. (e). The section of the structural fiber branched from the larger diameter fiber or fiber bundle. But note that the relation between the surface morphology and the mechanical properties was outside the scope of this research. 66

Figure 6.2.1.12. A backscattered electron image showing the fibers with different morphology. A section of a structural fiber, (a) branched from the larger diameter fiber or fiber bundle, (b). The fiber indicated by (b) was more chemically extracted than (c) because the fiber indicated by (c) had more calcium oxalate plates and the surface morphology of pit was more obvious than or

different from (b). The relation between the extent of chemical extraction and the surface morphology was only a speculation and it was uncertain whether the post-chemical extraction handling process and the number of types of fiber would affect the surface morphology. 67

Figure 6.2.2.1. A backscattered electron image with a larger magnification zoomed in on Figure 6.2.1.12. The sample was a chemically extracted fiber. Label 1 – 7 indicated the detection focus areas (with red sides) of the energy-dispersive X-ray spectroscopy. Label 2 focused on the calcium oxalate plate; label 7 focused on the remainder of the calcium oxalate plate; label 1, 5, and 6 focused on the pits; label 3 and 4 focused on the non-plate areas. 69

Figure 6.2.2.2. EDS results for label 1 (the pit) in Figure 6.2.2.1. 70

Figure 6.2.2.3. EDS results for label 2 (the calcium oxalate plate) in Figure 6.2.2.1. 71

Figure 6.2.3.1. A photo showing different parts of Typha leaves before chemical extraction. Left: lamina; middle: sheath; right: sheath with the ventral and dorsal surface removed. 72

Figure 6.2.3.2. A photo showing fibers extracted from different parts of Typha leaves by chemical extraction. Left: fibers from lamina; middle: fibers from sheath; right: fibers from sheath with the ventral and dorsal surface removed. Different colors could be observed. 73

Figure A2.1. EDS results for label 1 (the plate) in Figure 4.2.3.1. 96

Figure A2.2. EDS results for label 2 (the plate) in Figure 4.2.3.1. 97

Figure A2.3. EDS results for label 3 (the plate) in Figure 4.2.3.1. 98

Figure A2.4. EDS results for label 4 (the plate) in Figure 4.2.3.1. 99

Figure A2.5. EDS results for label 5 (the plate) in Figure 4.2.3.1. 100

Figure A2.6. EDS results for label 6 (the plate) in Figure 4.2.3.1. 101

Figure A2.7. EDS results for label 7 (the plate) in Figure 4.2.3.1. 102

Figure A2.8. EDS results for label 8 (the non-plate place) in Figure 4.2.3.1. 103

Figure A2.9. EDS results for label 9 (the non-plate place) in Figure 4.2.3.1. 104

Figure A2.10. EDS results for label 10 (the non-plate place) in Figure 4.2.3.1. 105

Figure A2.11. EDS results for label 11 (the non-plate place) in Figure 4.2.3.1. 106

Figure A2.12. EDS results for label 12 (the non-plate place) in Figure 4.2.3.1. 107

Figure A2.13. EDS results for label 13 (the non-plate place) in Figure 4.2.3.1. 108

<i>Figure A3.1. EDS results for label 1 (the pit) in Figure 6.2.2.1.....</i>	<i>109</i>
<i>Figure A3.2. EDS results for label 2 (the calcium oxalate plate) in Figure 6.2.2.1.....</i>	<i>110</i>
<i>Figure A3.3. EDS results for label 3 (the non-plate place) in Figure 6.2.2.1.</i>	<i>111</i>
<i>Figure A3.4. EDS results for label 4 (the non-plate place) in Figure 6.2.2.1.</i>	<i>112</i>
<i>Figure A3.5. EDS results for label 5 (the pit) in Figure 6.2.2.1.....</i>	<i>113</i>
<i>Figure A3.6. EDS results for label 6 (the pit) in Figure 6.2.2.1.....</i>	<i>114</i>
<i>Figure A3.7. EDS results for label 7 (the remainder of the calcium oxalate plate) in Figure 6.2.2.1.....</i>	<i>115</i>

CHAPTER 1. Introduction

1.1 Cattails and their interaction with wildlife

Cattails are a common plant that can be found in many places such as roadside ditches, lakes, estuaries, marshes, rice fields, and wet meadows. Certain living conditions may favor some species more than the others (Smith 1967). *Typha latifolia*, *T. angustifolia*, *T. x glauca*, and *T. domingensis* are a few *Typha* species, which could be seen often as the focus in the cattail plant-related research field. The common name “cattail” was used to refer to the entire genus *Typha* in the studies frequently (Grace and Harrison 1986).

Cattails have a lot of interactions with the environment and wildlife. The correlation of it with the living things can be positive and negative. Cattail rootstocks are valuable food for some wildlife. For example, starchy underground stems for geese and muskrats. Besides, cattails can also be a shelter and nesting cover. For example, yellow-headed blackbirds, red wings, and long-billed marsh wrens were found to nest in cattail marshes regularly (Martin et al. 1961).

However, the invasion of cattail affected the biodiversity conservation by degrading the communities of native plant and lowering the resources for wetland wildlife (Anderson et al. 2019). Besides, plots with cattails mowed were found to have more aquatic beetles than the intact plots in a freshwater marsh (Molnár et al. 2011).

1.2 Weed problem

Typha plants have increased in their distribution and abundance in the wetland ecosystems worldwide, especially in North America. The anthropogenic-related disturbances on wetland hydrology and nutrient loads caused this phenomenon, while non-native and hybrid taxa have

aggravated the *Typha* spreading. Disturbance to vegetation with eutrophication could promote the invasion of *Typha* on other plant communities. For example, hydrologically-disturbed and nutrient-enriched environment in dikes. The nutrient and hydrologic connection between wetlands and lakes were cut and therefore domination by *Typha* plants would occur. There are direct and indirect mechanisms for understanding *Typha*'s invasive characteristics. *Typha* directly has relatively higher productivity and more efficient excess nutrients utilization, while the accumulation of standing-dead leaf litter of *Typha* stands can indirectly harm other native species, but this effect is generally little for *Typha*'s growth. These mechanisms contribute to an easier *Typha* domination among the plant communities. Besides, it is worth mentioning that the increased soil inorganic nutrient availability can assist their reinvasion after herbicide application. *Typha* invasion can bring about more sediment deposition in wetlands and more nitrogen and phosphorus can enter the concerned biosystems. After the *Typha* plants are treated with herbicide, there will be a decreased plant demand for the nutrients and the availability of nutrients will increase, which provides chances for reinvasion, algal booms, or release of nutrients to adjacent aquatic systems. Oligotrophic downstream ecosystems could also result. Places affected by cattail plant is not only restricted to wetland. Indeed, industrial-scale agriculture, small-scale production, and subsistence farming can also be affected. In addition, *Typha* is known to block the water flow of some places. For instance, rivers, reservoirs, and drainage ditches (Bansal et al. 2019). The economic loss suffered from cattails can also be in an indirect relationship. It was found that sunflower fields have experienced more damage when they were close to cattail wetlands. Blackbirds have taken cattails as their habitat and targeted the sunflowers, which were near the wetland roost sites as their main source of food (Linz and Homan 2011).

1.3 Scientific application - phytoremediation

There are undoubtedly negative impacts which *Typha* plants can bring to the environment and can be related to their invasive characteristics. From the aforementioned cases about how living things have been affected by cattail, we can see the reasons why *Typha* can easily dominate among other plant species are mainly about their high tolerance for their growing conditions and their effective utilization of the available nutrients. *Typha* plants have negative effect in some aspects when they are not restricted. But they can still be applied strategically and productively.

Typha was researched broadly in the phytoremediation area. Phytoremediation means to make use of plants and their growing process to detoxify organic and inorganic pollutants. The xenobiotics in the contaminated environment are extracted and/or mineralized by them, while depending on the tolerance of them for the contaminants (Wilson et al. 2000). Researchers have attempted to use *Typha* for different types of contaminants. For example, *T. latifolia* was able to demonstrate the capability to remove nitrogen and phosphorus composition in the urban wastewater which came from a sewage plant to a downhill pilot system (Leto et al. 2013).

In fact, nutrients are not the only substances that cattails can help remove. Some heavy metals are also their target. *T. latifolia* could extract Pb, Cd, Cr, Mn, and Fe from the water surroundings and mainly the roots were found to have higher heavy metal concentrations than the stem and leaves (Carranza-Álvarez et al. 2008). It was also reported that *T. angustifolia* can uptake Cr by surface layer absorption and transportation. The heavy metal stresses can be lessened by thickening the cell walls or secreting chemical substances. (Chen et al. 2014).

Besides, cattail could also be useful for treating contaminants other than nutrients and heavy metals. It was found that *T. latifolia* demonstrated possibility for remediating the pesticide-contaminated rinsates with the research focus being put on metalaxyl and simazine (Wilson et al.

2000). *T. Angustifolia* was also studied for its capability to treat synthetic reactive dye wastewater under caustic conditions. The pH could be lowered and the color and sodium could be removed to a certain extent (Nilratnisakorn et al. 2007).

The various types of contaminants that *Typha* plants are able to extract have showed their application in the environmental area. If their properties are further investigated, wetland treatment may become a common option for some polluted areas because of their potentially cost-effective nature. Nevertheless, their environmental amelioration function does not cancel out their negative effects, which could be observed in their natural habitat. Therefore, rigorous management is certainly required for *Typha* plant-related phytoremediation.

1.4 Other scientific applications - *Typha* fibers

The tough survival characteristics of *Typha* are not only based on their effective utilization of nutrients. Indeed, the environmental factors are not only limited to nutrients and species rivalry, but also the physical aspect, such as their resistance to air current. Their fibers are very important constituents in their framework for bracing the cattail. This has provided a lot of potentials in the research areas which were related to their mechanical properties. For instance, cattail fibers were studied for a few properties to see their suitability as insulation boards and the results suggested that insulation boards could be made by narrow-leaved cattail fibers with a binder with energy saving and environmentally friendly benefits (Luamkanchanaphan et al. 2012). Cattail fibers were also studied as binder-free fiber reinforced composites. The research outcome showed good mechanical properties with the self-gluing characteristic and surface properties with potential applications in automotive and furniture industries (Wuzella et al. 2011).

Research has shown that cattail fiber can be extracted chemically by using alkaline chemicals (Chakma et al. 2018; Sana et al. 2014). Cattail leaf fiber extraction has been investigated for an optimized extraction protocol in hopes of producing fibers with high quality for different aspects such as yield percentage, moisture regain, tensile strength, etc. If it is successful, cattail can be a very attractive source for fibers because of its growing characteristics with high adaptability. If other sources of fiber involve more demanding plantation conditions, cattail may be a lower cost alternative. But the feasibility for introducing it to the industry would require more scrutinization in order to determine whether using it for a certain application will not harm the environment, while at the same time its quality has to be comparable to other common fibers and it has to have a lower production cost.

Researchers were also interested in examining the oil sorption application of cattail seed fiber assemblies. Dong et al. (2015) blended seed fibers with polymers to make fiber assemblies. Seed fibers were blended with 10 – 20 % polypropylene/polyethylene sheath-core composite fiber, carded into fibrous web, and the fibers in the web were thermally bonded. The samples were taken to test different oil and water sorption-related properties.

While efforts have been made in finding out how cattail fiber can be applied in different fields, it is worth noticing that the definition of “fiber” actually can have different meanings in different research. Cattail fiber is not restricted to the leaf fiber only and leaf fiber itself can also have different meanings. Therefore, when reading a scientific report, journal article, review, etc., it is important to pay attention to a few matters in order to understand the focus of their research. Firstly, the species. For example, *T. latifolia*, *T. angustifolia*, *T. domingensis*, etc., while some might just refer to *Typha* spp. Secondly, the type of fiber. For example, leaf fiber and seed fiber. Thirdly, the characteristics. For example, the morphological definition of leaf fiber. For the

experimental results and analyses in this thesis, the species of the cattail was *Typha* without further classification and the term “fiber” (or its plural form) was defined in section 2.2.

1.5 Objectives

Typha leaf fibers have potential to become a new type of common fiber in industry. The development of *Typha* leaf fibers has not reached an industrial level as its feasibility for industrialization and areas of application remain uncertain. Many studies on *Typha* leaf fiber extraction had a focus on the chemical approach by using an alkaline solution as a main reagent. Indeed, inside the *Typha* leaf, there were a lot of lacunae and across these lacunae, there were lacunae fiber cables (and in this thesis they were called lacunae fibers). In the plant science area, scientists have demonstrated the possibility to extract them manually (Witztum and Wayne 2014, 2015, 2016a, 2016b).

The first objective of this research was to develop a manual extraction method to extract *Typha* leaf lacunae fibers. Knowledge from plant science literature was applied to the fiber engineering field by designing a preliminary extraction method, which might be beneficial for the further development of *Typha* leaf fiber extraction.

The second objective was to investigate the morphology of the manually extracted fibers and chemically extracted fibers in order to better understand the fiber products in the extraction.

The third objective was to compare the manual and chemical approach for *Typha* leaf fiber extraction with respect to the differences of the extraction processes and fiber products.

CHAPTER 2. Literature review

2.1 Botanical studies on the *Typha* leaf structure

Typha plants have noticeable leaf structures, which allow them to confront the difficult environmental conditions. Research has been conducted on different species of *Typha* plant, trying to understand how their structures were related to their survival and development. The leaf of *Typha latifolia* was like a light weight cantilever beam mounted vertically at its lower end and there was a large surface area provided by the sandwich-type construction for carrying out photosynthesis on a metabolically economical core (Rowlatt and Morshead 1992). The leaf blade of *Typha* could intercept photosynthetically active radiation. Some of the internal tissue of the photosynthetic organ broke down to form lacunae. Some parts of the *Typha* were submerged. Lacunae were needed for oxygen to flow from the leaf to the submerged parts and carbon dioxide to flow from the submerged parts to the photosynthetic parts of the *Typha* plant. Other tissues included the dorsal and ventral tissue, diaphragms that consisted of two or three layers of stellate cells of aerenchyma tissue, fiber cables that traversed the diaphragms and lacunae, and partitions that separated the dorsal and ventral tissue (Witztum and Wayne 2014). Note that this thesis considered the term “leaf blade” same as “lamina”. For *Typha latifolia*, the transport of oxygen through the aerenchyma minimized the anerobic respiration in the below-ground tissue. Reduced and potentially toxic compounds in sediments surrounding the below-ground tissue might also be oxidized as well (Constable and Longstreth 1994). According to Witztum and Wayne (2014), polarized light microscopy showed that the cellulose microfibrils of the fiber cable walls were oriented in a direction parallel to the fiber long axis. This orientation was important for the characteristics of the fiber cables that they were mechanically stiff and strong under tension. The

fiber cables together with other leaf components formed a tensegrity structure. It created load paths through which stresses could be redistributed throughout the 1 – 3 m tall upright leaves of *T. latifolia*, *T. angustifolia*, *T. x glauca*, *T. domingensis*, and *T. shuttleworthii*.

Lacunae fibers, which were called as fiber cables in literature were the target fiber of the proposed manual extraction method. Photos of the structures of *Typha* leaves can be seen in chapter 4.

2.2 Definition of a fiber

Defining a fiber is very important in this project especially when the comparisons of fibers were made. Groups who work on the natural fiber subject may have a clear view on their requirements which may be different from others. For example, the textile manufacturer and papermaker could have different consideration. For a textile fiber, the length to width ratio would be in excess of 10^3 , but for paper fibers, it would usually be less than 10^2 (McDougall et al. 1993).

This research utilized the minimum length/diameter ratio in excess of 10^2 as the only criteria for considering a material as a fiber. Other properties were not included in the criteria because the exact application of cattail leaf fibers in this research was not clear.

2.3 Chemical extraction of *Typha* fibers

Some research groups (Chakma et al. 2018; Sana et al. 2014) would use a similar protocol to obtain the *Typha* leaf fibers. The collected leaves were immersed in sodium hydroxide solution or potassium hydroxide solution at a certain concentration and temperature to remove a considerable portion of leaf material. People have researched different parameters such as temperatures and chemicals. Different parameters could produce fibers with different quality and

yield percentage. After that, the fiber products could be neutralized by using water or dilute acetic acid. The products could be washed by water after each procedure. This protocol might induce two problems, which were not advantageous for the further development of *Typha* leaf fiber. Firstly, the costs of chemicals and wastewater treatment would be higher because the required amount of alkaline solution with other costs were for the entire extraction process of fibers rather than only changing the surface morphology of fibers. Secondly, the rationale to extract fibers using the chemical approach was to remove the structural materials and dissociate the leaf structures in order to obtain the fibers. The extraction conditions need to be optimized in order to produce fiber products with more regular properties, such as fiber diameter and structural morphology. If there is an extraction method which can directly extract cattail leaf fibers from the structure of the *Typha* leaf and potentially with a lower cost in some aspects, it can be more research and industrial-friendly.

2.4 *Typha* fibers diameter

Chakma et al. (2018) investigated the fiber diameter from the fibers extracted from *Typha* plants under different chemical extraction conditions. The chemical concentration was set as 3%, liquor ratio was constant at 1:50, and the samples were the mixture of different plant types: with a hard stem, with a soft stem, and with no stem. Different chemicals were tried: potassium hydroxide and lithium hydroxide. Experiments with different extraction time included 2, 4, 6, and 8 hours. Temperatures for extraction were 80°C (for KOH solution and LiOH solution) and 95°C (for KOH solution). Apart from that, extraction condition of 3% NaOH, 80°C, and 4 hours was also tried. According to their data obtained from random sampling of *Typha* fiber, the resulting mean fiber diameters ranged from 37.67 μm to 107.47 μm , while the CV% ranged from 36.38% to 105.39%.

A relatively small mean diameter and at the same time low CV% from these products was $46.27 \pm 16.83 \mu\text{m}$ (mean $\pm$ SD) with the CV%, 36.38%.

Sana et al. (2014) investigated the *Typha* leaf fibers and the extraction conditions were liquor ratio: 1/40, sodium hydroxide solution, different extraction time: 2, 3, and 4 hours, different temperatures: 80°C, 100°C, and 120°C, and different concentrations: 10 g/L, 20 g/L, and 30 g/L. The mean diameters of the products ranged from 141 μm to 506 μm .

A relatively larger diameter and variation was a problem and could limit the application. However, lacunae fiber cables, which were inside the leaves were found to have relatively regular diameters and variations. The lacunae fiber cables of *T. x glauca* leaf blade were measured to have diameters of $35 \pm 5 \mu\text{m}$ (mean $\pm$ SD, n = 25) with the diameter range being approximately 20 μm – 60 μm (Witztum and Wayne 2014). The CV% could be calculated to be about 14.29%. The lacunae fiber cables of *T. angustifolia* were also measured to have diameters of $18.55 \pm 3.34 \mu\text{m}$ (n = 19) for leaf blade and $55.52 \pm 18.47 \mu\text{m}$ (n = 31) for leaf sheath (mean $\pm$ SD, n = 25) (Witztum and Wayne 2016b). The CV% could be calculated to be about 18.01% (leaf blade) and 33.27% (leaf sheath).

This research aimed to develop an extraction method to specifically extract the lacunae fiber cables from the leaf sheath.

CHAPTER 3. Materials and methods

3.1 Materials

The *Typha* plants were collected in Manitoba. The exact species might not be the concern so long as there were lacunae fibers inside the leaf of that *Typha* species and the steps in the method were applicable to its structure. Since there were a lot of details pertaining to the manual extraction approach, the description of the method was put in chapter 4. The rationale of the manual extraction method was to selectively and physically remove the surrounding leaf materials and separate the lacunae fibers from the leaf structure.

3.2 Chemicals

The potassium hydroxide solution was prepared by adding potassium hydroxide pallet (Fisher Chemical) to water.

Acetic acid solution was prepared by adding acetic acid (Fisherbrand® ; glacial) to water.

3.3 Chemical extraction and chemical treatment

The samples for the chemical extraction and chemical treatment were put in beakers with potassium hydroxide solution. For the experiment of chemical extraction and chemical treatment at higher temperature (70 – 80°C), the beakers with samples and potassium hydroxide solution were put in hot water baths and heated for 2 hours.

Typha leaves floated and many of them were not totally immersed in the alkaline solution. Parts of the leaves would be less extracted. Although some (but not all) less extracted parts of the samples were cut and discarded in the post-extraction process, the remaining samples, which were

investigated could also be extracted in different extent. Therefore, this could be one of the reasons that caused the *Typha* leaves to have different extent of extraction in a batch of sample.

3.4 Diameter measurement

Two methods were used to measure the diameters of fibers. The first method was to use an optical microscope, which was connected to a camera and computer. Using a software of Bioquant, three places in a fiber (excluding the nodes) were chosen to measure the diameters, the average of them was taken, and this was considered as one sample.

The second method was to measure the diameters using the ESEM images of the fibers and the software used for measurement in this method was ImageJ (v1.53e). Three places in a fiber (excluding the nodes) were chosen to measure the diameters, the average of them was taken, and this was considered as one sample.

It is important to note that in this research a lacunae fiber could be cut into more than one fiber with shorter lengths and they were considered as different samples in the diameter measurement. This might result in having different diameter measurement data (mean, SD, CV%, and variance) and different conclusions than measuring lacunae fiber samples strictly not from the same fiber.

3.5 Environmental scanning electron microscopy (ESEM) imaging

FEI Quanta 650 FEG was used to examine the morphology of the *Typha* leaves and fibers. The samples were mounted on aluminum specimen stubs by using double-sided tape. After that, they were put into the ESEM.

3.6 Energy-dispersive X-ray spectroscopy (EDS) analysis

Octane Super SDD from EDAX was attached to the system for the EDS analysis. The software TEAM™ was used to collect and analyze the EDS data.

3.7 Statistical analyses

RStudio software (RStudio Team 2016) has been used for the statistical analyses.

CHAPTER 4. Manual extraction of lacunae fibers

4.1 Manual extraction method

In this section, the proposed manual extraction method for *Typha* leaves was explained. The target fibers in the extraction process were the lacunae fibers. This extraction method was suitable to the sheath or the lowest part of a lamina, but not suitable to the middle to upper part of a lamina (lamina has the same meaning as leaf blade in this thesis). This method was carried out manually and assisted with tools. 4 steps were shown here to extract the lacunae fibers. Note that these steps and images were only for explaining the feasibility of extracting the lacunae fibers from the *Typha* leaf structure. There were more technical steps and skills in the actual extraction process. Besides, not all images (photos and ESEM images) were taken from the same leaf.

Step 1 – leaf compression, removal of the thinner parts of the leaf at both sides, and removal of the dorsal surface

A *Typha* leaf has dorsal and ventral surface. The concave shape could be observed at the sheath. The green arrows in Figure 4.1.1. were indicating the two surfaces.

Before or after compressing the *Typha* leaf, the thinner parts of the leaf at both sides could be removed, which was indicated by the green lines (into the paper) on Figure 4.1.2. Flatten the leaf by compressing it, which was indicated by the purple arrows on Figure 4.1.2. After that, the dorsal surface was cut. The starting position was indicated by the red dashed line (into the paper) on Figure 4.1.2, which was supposed to be straighter after the compression. The cutting direction was along the leaf longitudinally, which was indicated by the yellow arrow on Figure 4.1.3. The ESEM image, Figure 4.1.4 showed the cross-section of a *Typha* leaf.

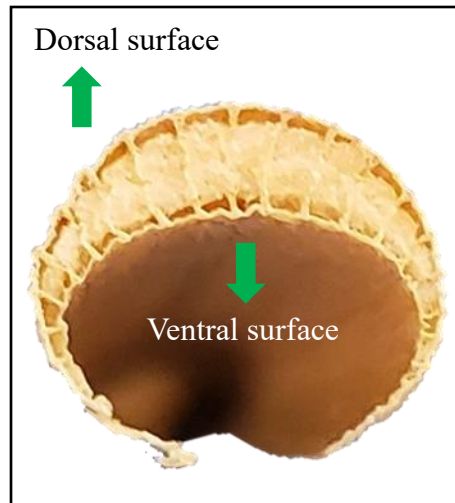


Figure 4.1.1. Cross-section of a Typha leaf.

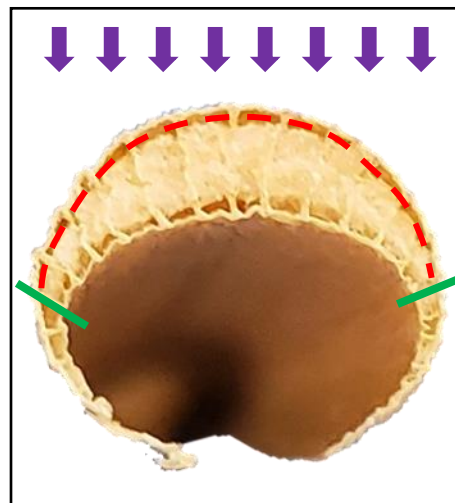


Figure 4.1.2. Before or after compressing the Typha leaf, the thinner parts of the leaf at both sides could be removed, which was indicated by the green lines (into the paper). Compress the Typha leaf as shown by the purple arrows and cut the dorsal surface according to the red dashed line (into the paper).

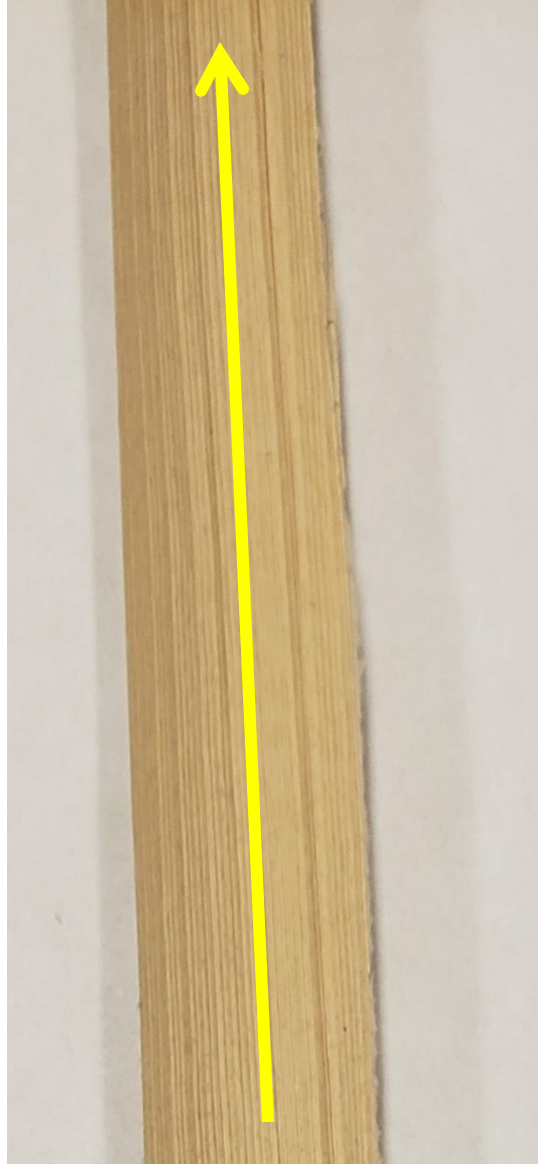


Figure 4.1.3. Top view of a Typha leaf. Cut the dorsal surface longitudinally. The yellow arrow indicated the cutting direction.

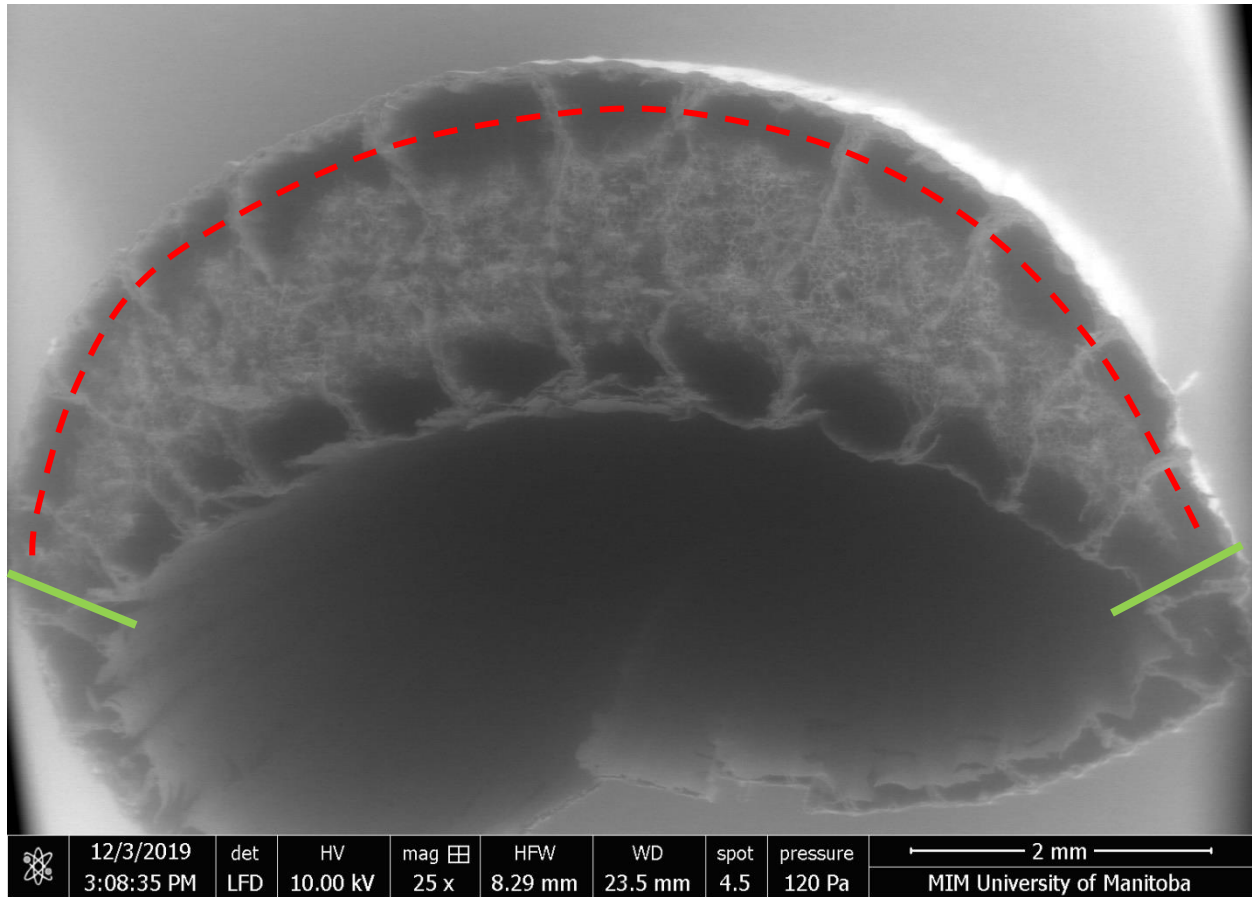


Figure 4.1.4. An ESEM image of the cross-section of a Typha leaf. Before or after compressing the Typha leaf, the thinner parts of the leaf at both sides could be removed, which was indicated by the green lines (into the paper). The red dashed line indicated the starting position to cut the dorsal surface (into the paper). The red dashed line should be straighter after the compression.

Step 2 – column cut

After the dorsal surface had been removed, the inner structure could be observed (Figure 4.1.5 and 4.1.6). This step was to cut the leaf and obtain the column. Four components were focused: the non-diaphragm aerenchyma (indicated by the blue arrow in Figure 4.1.7), lacunae fibers (indicated by the purple arrowheads in Figure 4.1.6 and 4.1.7), partitions (indicated by the brown arrowheads in Figure 4.1.6. and 4.1.7), and diaphragms (indicated by the black arrowheads at the right of the

red dashed line in Figure 4.1.6 and surrounded by orange dashed lines in Figure 4.1.7). The structures were cut according to the red dashed lines on Figure 4.1.6 and 4.1.7, which were beside the lacunae fibers. Note that, during the cut, it should be avoided to include the partitions because the main purpose of this step was to isolate a column without the partitions.



Figure 4.1.5. The top view of a Typha leaf with the dorsal surface removed.

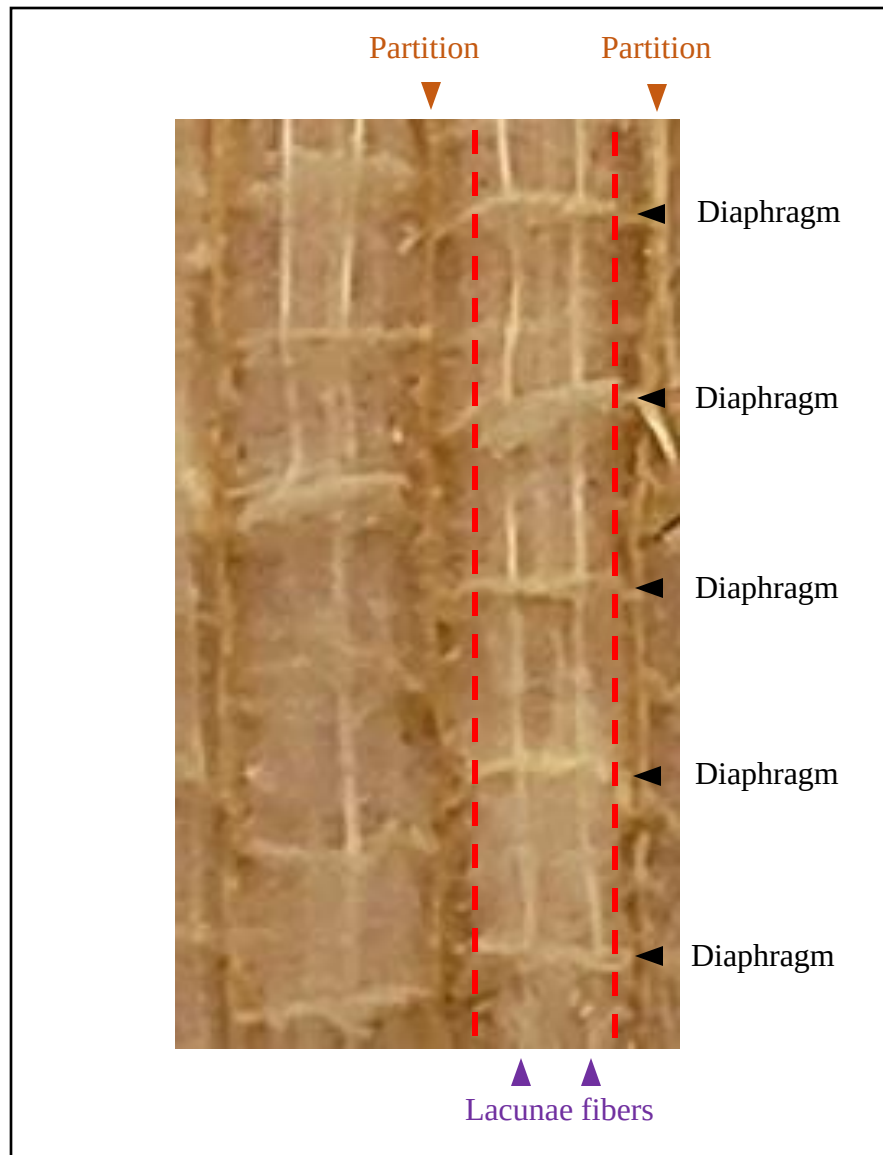


Figure 4.1.6. The top view of a Typha leaf with the dorsal surface removed. Brown arrowheads on the top indicated the partitions. Black arrowheads at the right of the red dashed line indicated the diaphragms. Purple arrowheads at the bottom indicated the lacunae fibers. A column could be separated by cutting along the red dashed lines.

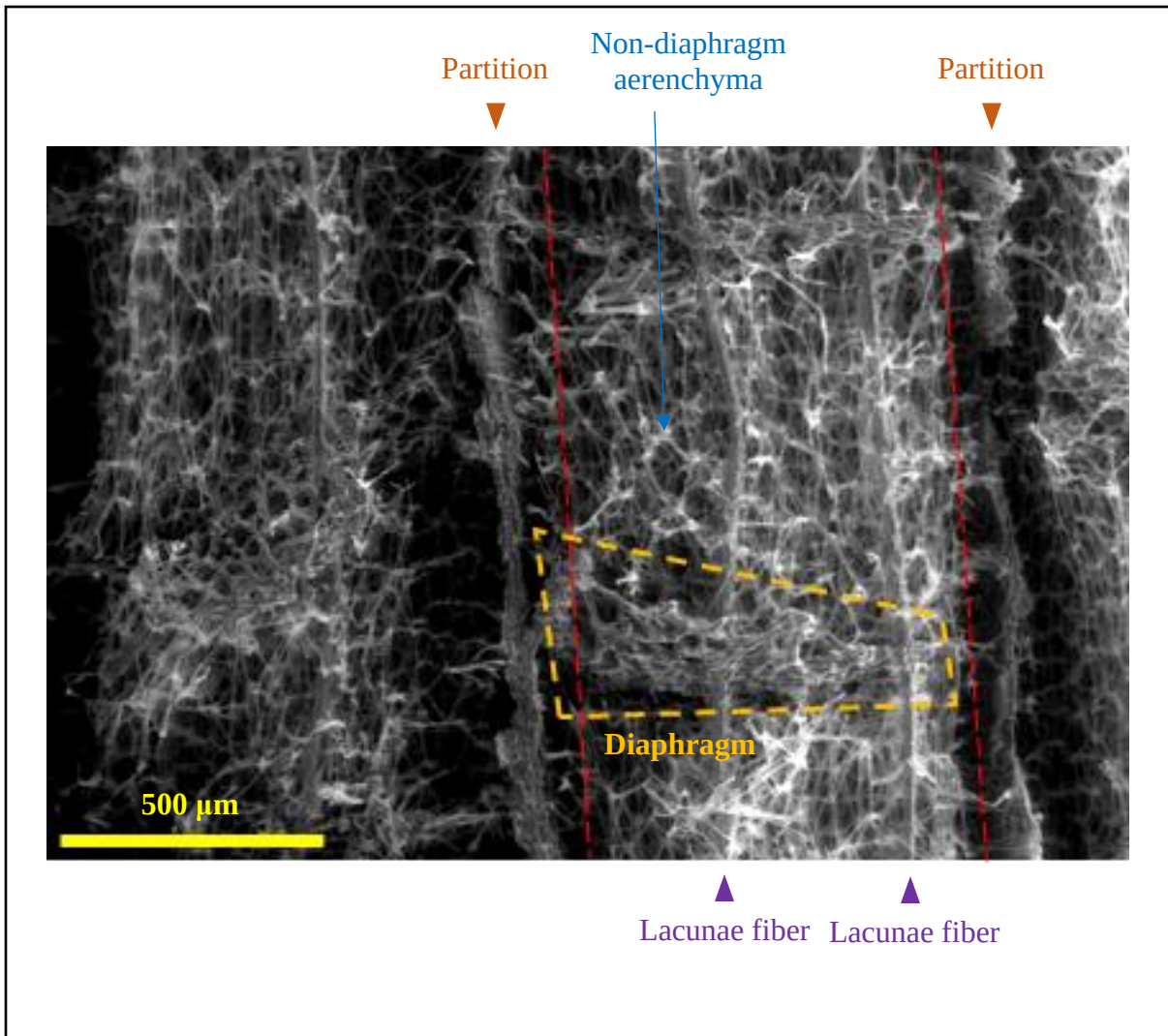


Figure 4.1.7. An ESEM image of the top view of a *Typha* leaf with the dorsal surface removed. The blue arrow indicated the non-diaphragm aerenchyma. The brown arrowheads on the top indicated the partitions. The area with orange dashed sides indicated the diaphragm. The purple arrowheads at the bottom indicated the lacunae fibers (the target fibers of the manual extraction method). A column could be separated by cutting along the red dashed lines. The yellow scale bar indicated 500 μm .

Step 3 – removal of the ventral surface

A column and the scaled up image was shown in Figure 4.1.8. Because the ventral surface had not been cut in step 1, then it was removed in this step (indicated by the red dashed line in Figure 4.1.8). The darker brown area in the scaled up image in Figure 4.1.8 was the ventral surface.

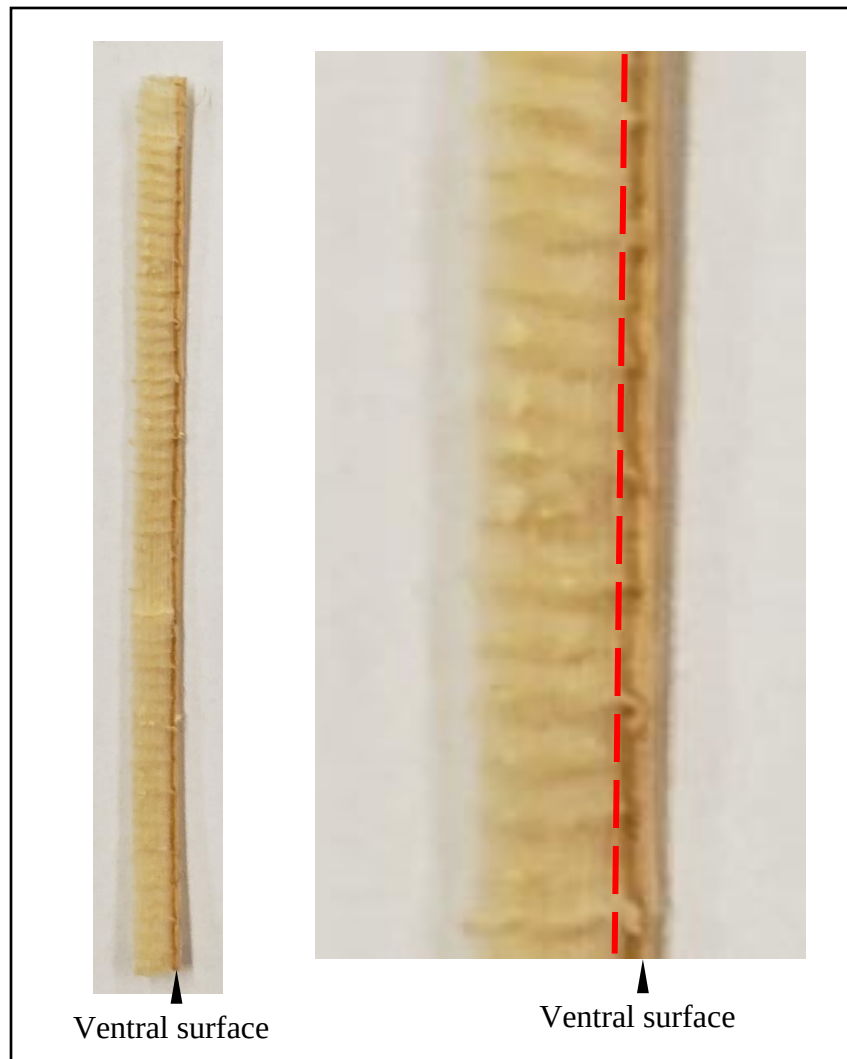


Figure 4.1.8. The image on the left was a shorter section of a removed column. It was the side view. The black arrowhead indicated the ventral surface. The image on the right was the scaled up image of the image on the left. The ventral surface could be separated by cutting along the red dashed line.

Step 4 – removal of the aerenchyma

The ventral surface was removed from the column in step 3 and was shown on the right in Figure 4.1.9 (indicated by the black arrowhead in Figure 4.1.9), while the column without the ventral surface was shown on the left in Figure 4.1.9 (indicated by the black arrowhead in Figure 4.1.9). A small section of the column (without the ventral surface) was pinched (indicated by the purple arrowheads in Figure 4.1.9) and pulled in the longitudinal direction (indicated by the blue arrow in Figure 4.1.9). This step was repeated for a few times until (most of) the aerenchyma surrounding the lacunae fibers was removed from the column and was repeated for the remaining unextracted section. Note that the nodes and calcium oxalate plates were found on the extracted lacunae fibers under a microscope after this step (this would be further discussed in section 4.2). Additional steps or methods were required to remove these types of surface morphology and confirm their removal, but the detailed investigation on their removal was outside the scope of this study.

Two columns which were partially extracted were shown in Figure 4.1.10. The difference between them was that the one on the left could not be easily extracted by a few pinching and pulling, but the one on the right could be extracted easily. The difficulty in the extraction of the column on the left was caused by the partition, while the column on the right did not contain or contained fewer partition materials and therefore an easier extraction process. This was the reason why avoiding the partition materials was important.

The extraction process was carried out manually and sometimes partition materials could remain on the column. Based on the experimental experience, pinching and then pulling these partition materials together with the column could break the samples, possibly due to the augmented force used. Even if the non-diaphragm aerenchyma, diaphragms, and some partition

materials were carefully removed, fibers or strand-like materials which looked different from the lacunae fibers could also be obtained, but the details of these fibers were not clear.

The ESEM image, Figure 4.1.11 showed a partly extracted column. The purple arrowheads in Figure 4.1.11 indicated the approximate place for pinching. Pinching and pulling the column for a long distance at a time might break the fibers. The fibers in Figure 4.1.11 could be seen with the place where the non-diaphragm aerenchyma and diaphragms were mostly removed. No obvious partition materials could be found in Figure 4.1.11. The extraction process could be easy without the hindrance of the partition. After the non-diaphragm aerenchyma and diaphragms were mostly removed, the extracted fibers could be washed by water at last. A bunch of extracted fibers were shown in Figure 4.1.12 and they were not only extracted from a single column.

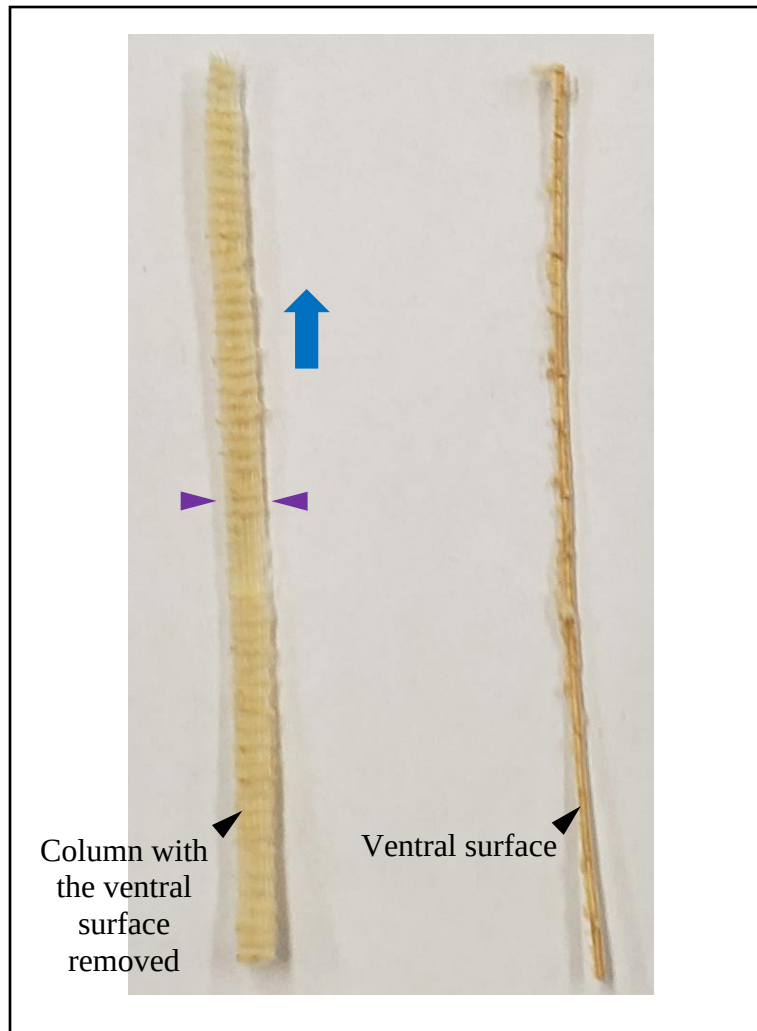


Figure 4.1.9. The substance on the left was the column, which had the ventral surface removed.

The purple arrowheads indicated the place for pinching. The blue arrow indicated the longitudinal direction for pulling after the pinching. The substance on the right was the ventral surface.



Figure 4.1.10. The column on the left contained partition materials, which caused a difficult extraction process; the column on the right did not contain or contained fewer partition materials and therefore an easier extraction process resulted.

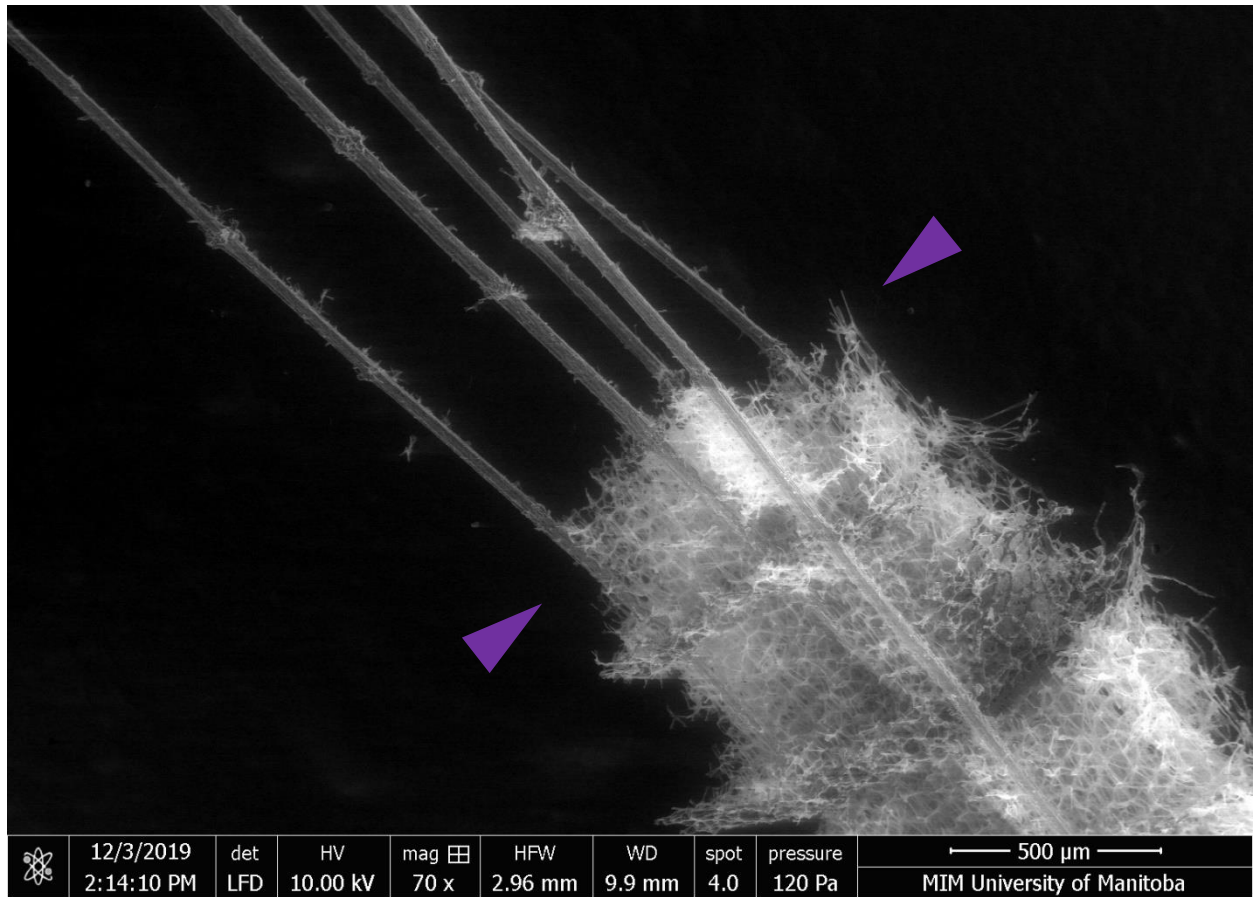


Figure 4.1.11. An ESEM image of a column with part of the non-diaphragm aerenchyma and diaphragms removed. The purple arrowheads indicated the approximate place for pinching.



Figure 4.1.12. A bunch of extracted fibers, which were not only obtained from a single column.

4.2 Characterization of the fibers

4.2.1 Morphology

Figure 4.2.1.1 showed a lacunae fiber obtained from the manual extraction method. Nodes and calcium oxalate plates were on the fiber, but calcium oxalate plates were more difficult to be observed in this image. Figure 4.2.1.2 showed a manually extracted lacunae fiber with a higher magnification. Calcium oxalate plates could be observed on the fiber. Figure 4.2.1.3 showed a manually extracted lacunae fiber with a higher magnification. A node and calcium oxalate plates could be observed on the fiber. Figure 4.2.1.4 showed a lacunae fiber anchored in a diaphragm with a partition next to the diaphragm. Figure 4.2.1.5 showed a partition with non-diaphragm aerenchymae at both sides of it. Figure 4.2.1.6 showed an enclosed lacunae fiber. Figure 4.2.1.7 showed an extracted lacunae fiber. Figure 4.2.1.1 – 4.2.1.7 were ESEM images. Based on these images, there were nodes and calcium oxalate plates on the manually extracted lacunae fiber.

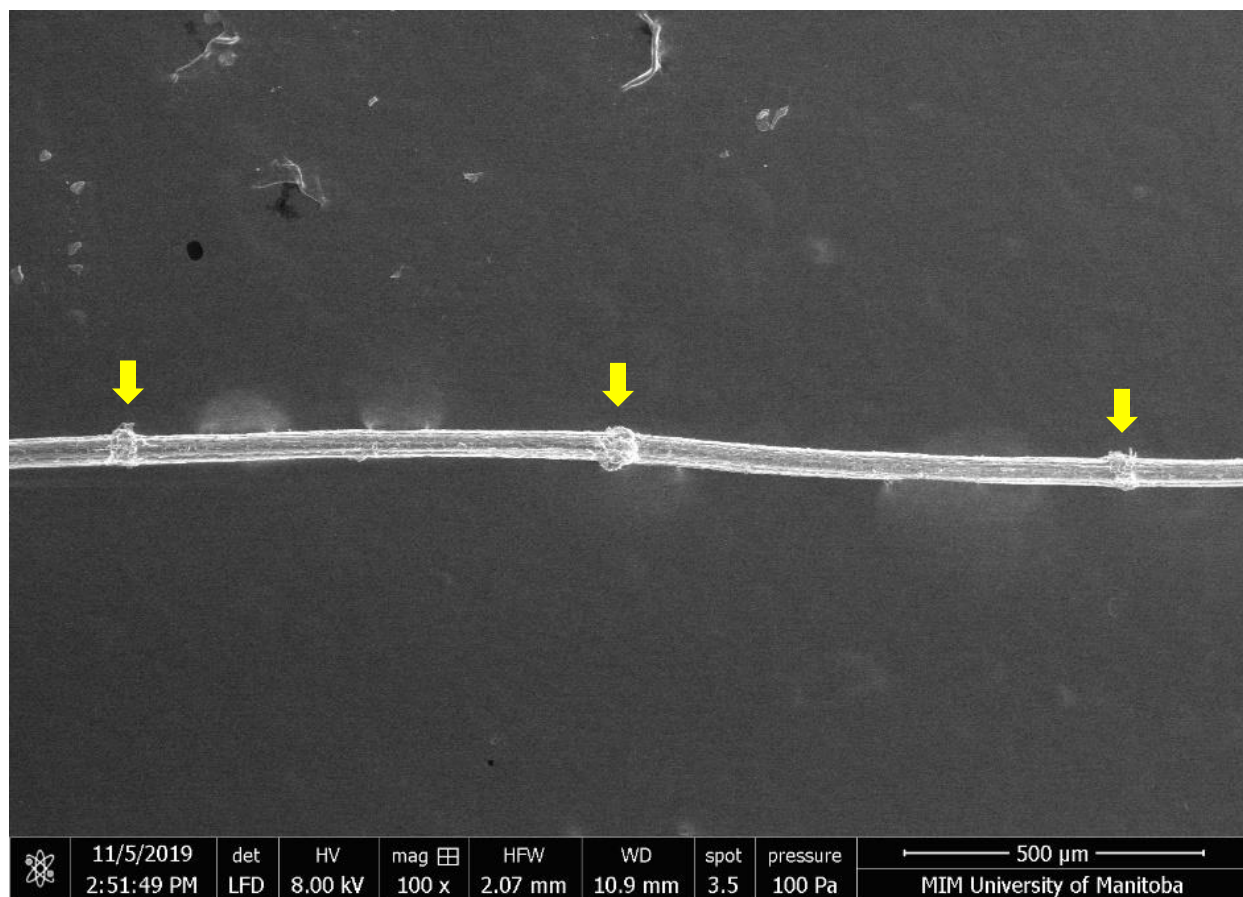


Figure 4.2.1.1. An ESEM image of a lacunae fiber obtained from the manual extraction method. Nodes (indicated by the yellow arrows) could be observed. There were calcium oxalate plates on this fiber, but they were difficult to be observed in this image.

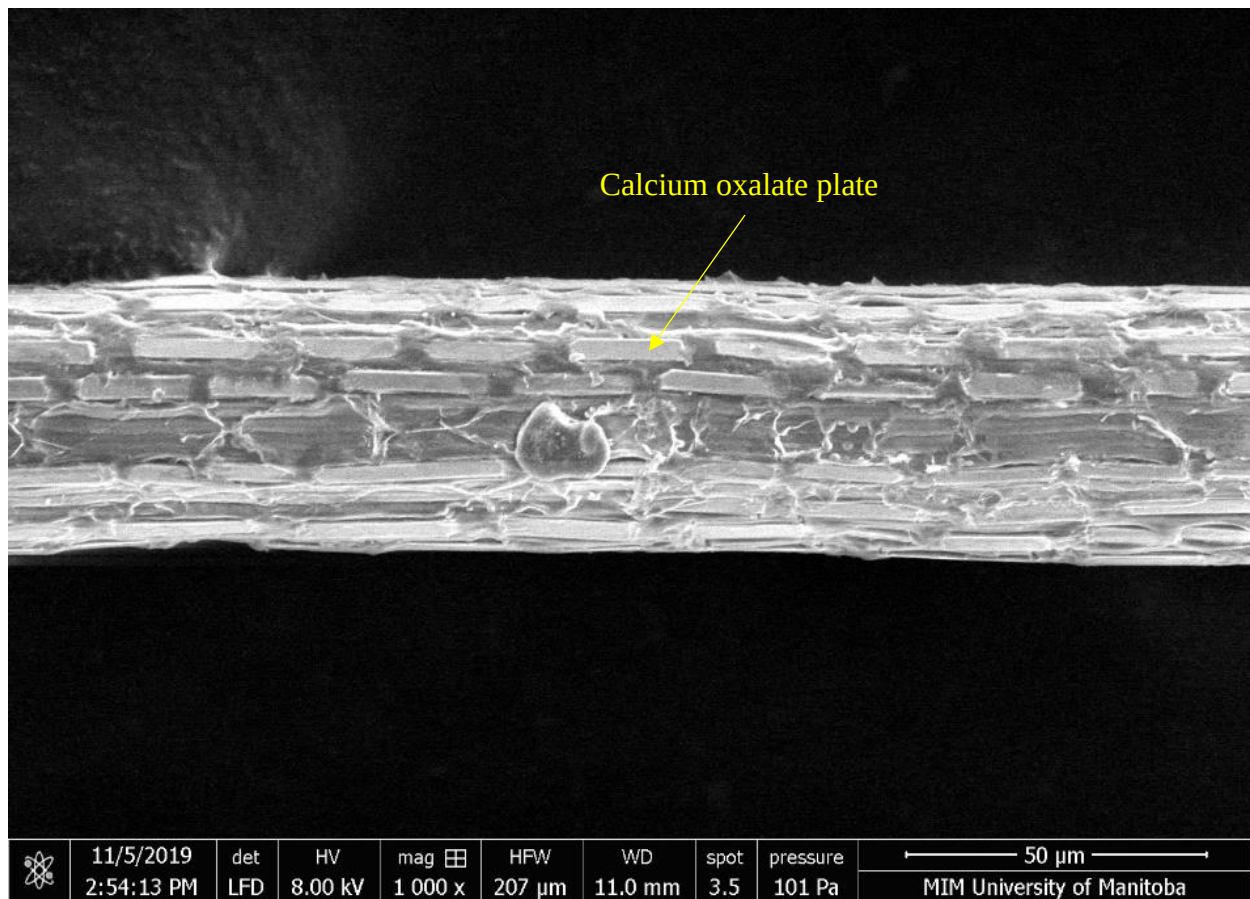


Figure 4.2.1.2. An ESEM image of a closer view of a manually extracted lacunae fiber. Calcium oxalate plates (an example was indicated by the yellow arrow) could be observed.

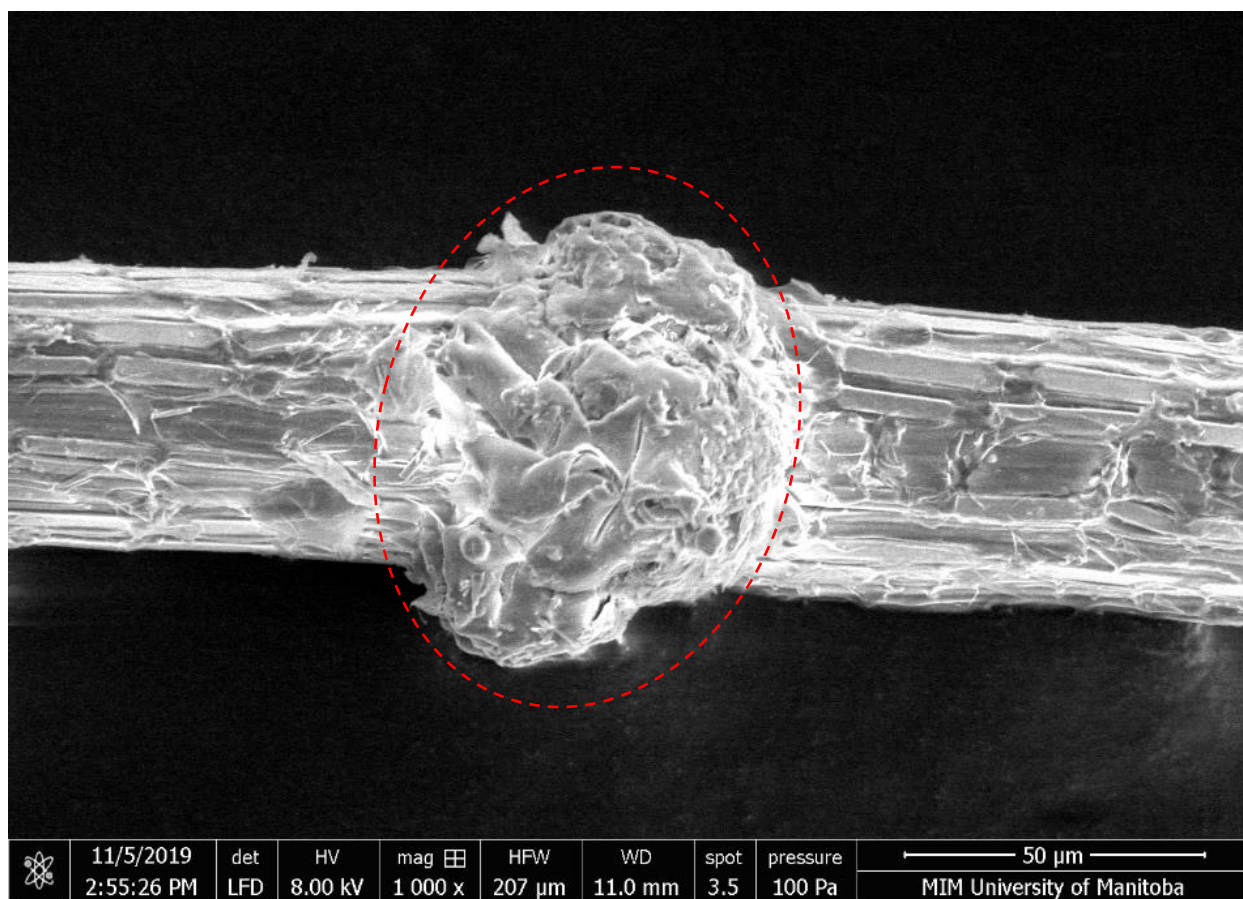


Figure 4.2.1.3. An ESEM image of a closer view of a manually extracted lacunae fiber. A node (indicated by the area with a red dashed side) and calcium oxalate plates could be observed.

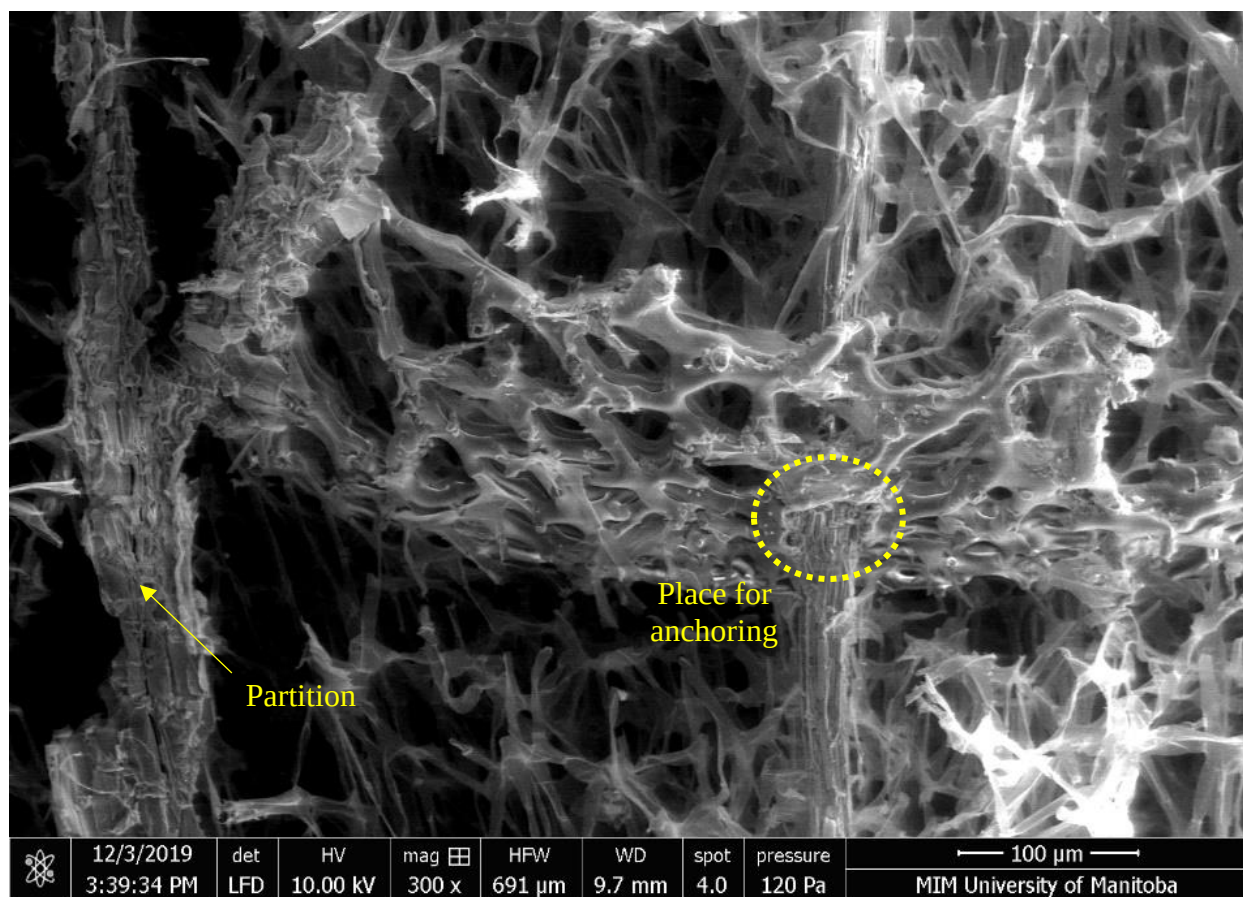


Figure 4.2.1.4. An ESEM image of a larger magnification of Figure 4.1.7, which showed a lacunae fiber anchored (indicated by the area with a yellow dotted side) in a diaphragm with a partition next to the diaphragm (indicated by the yellow arrow).

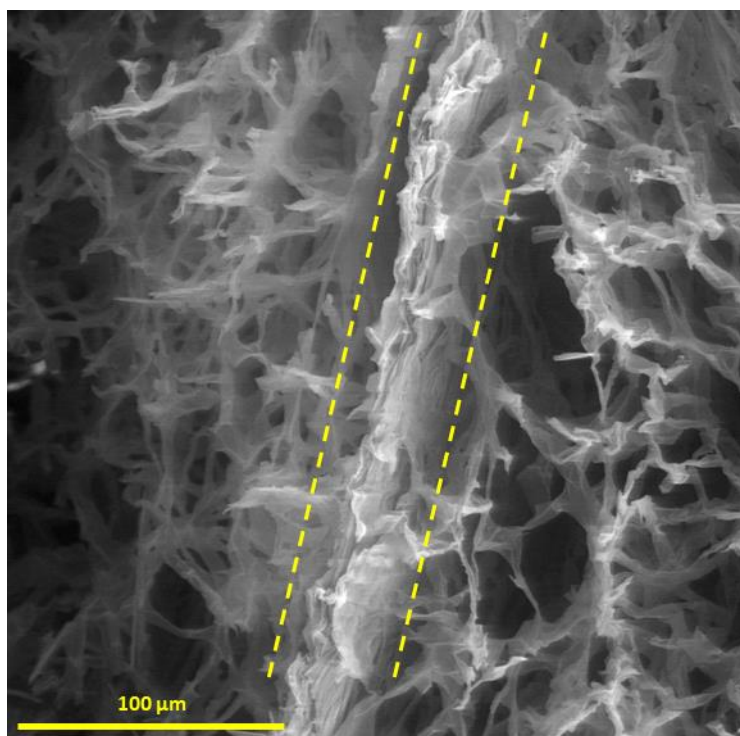


Figure 4.2.1.5. An ESEM image of the structure of a Typha leaf. The cross-section of the partition was between the yellow dashed lines. The non-diaphragm aerenchymae were at both sides of the partition. The yellow scale bar indicated 100 μm .

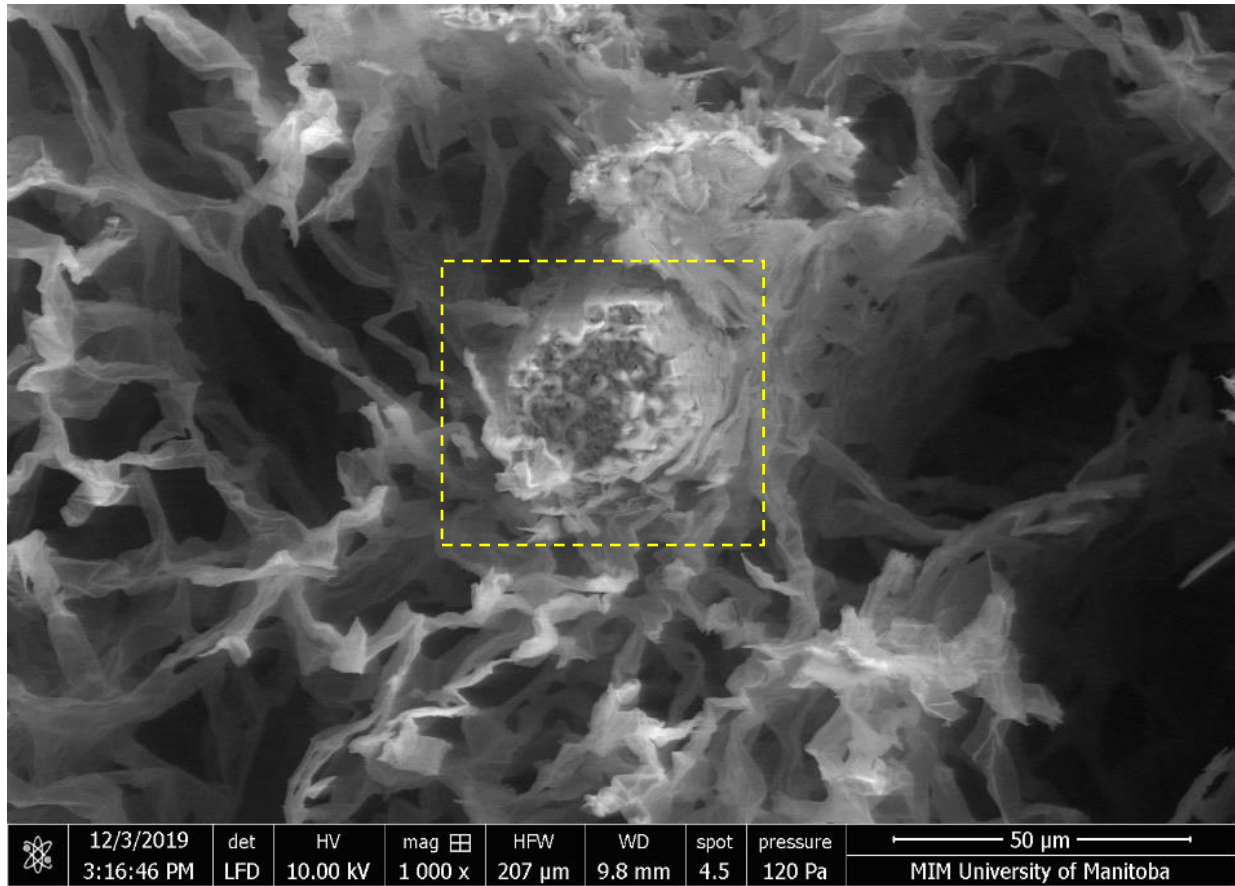


Figure 4.2.1.6. An ESEM image showing an enclosed *Typha* leaf lacunae fiber (indicated by the area with yellow dashed sides). The cross-section of it was shown.

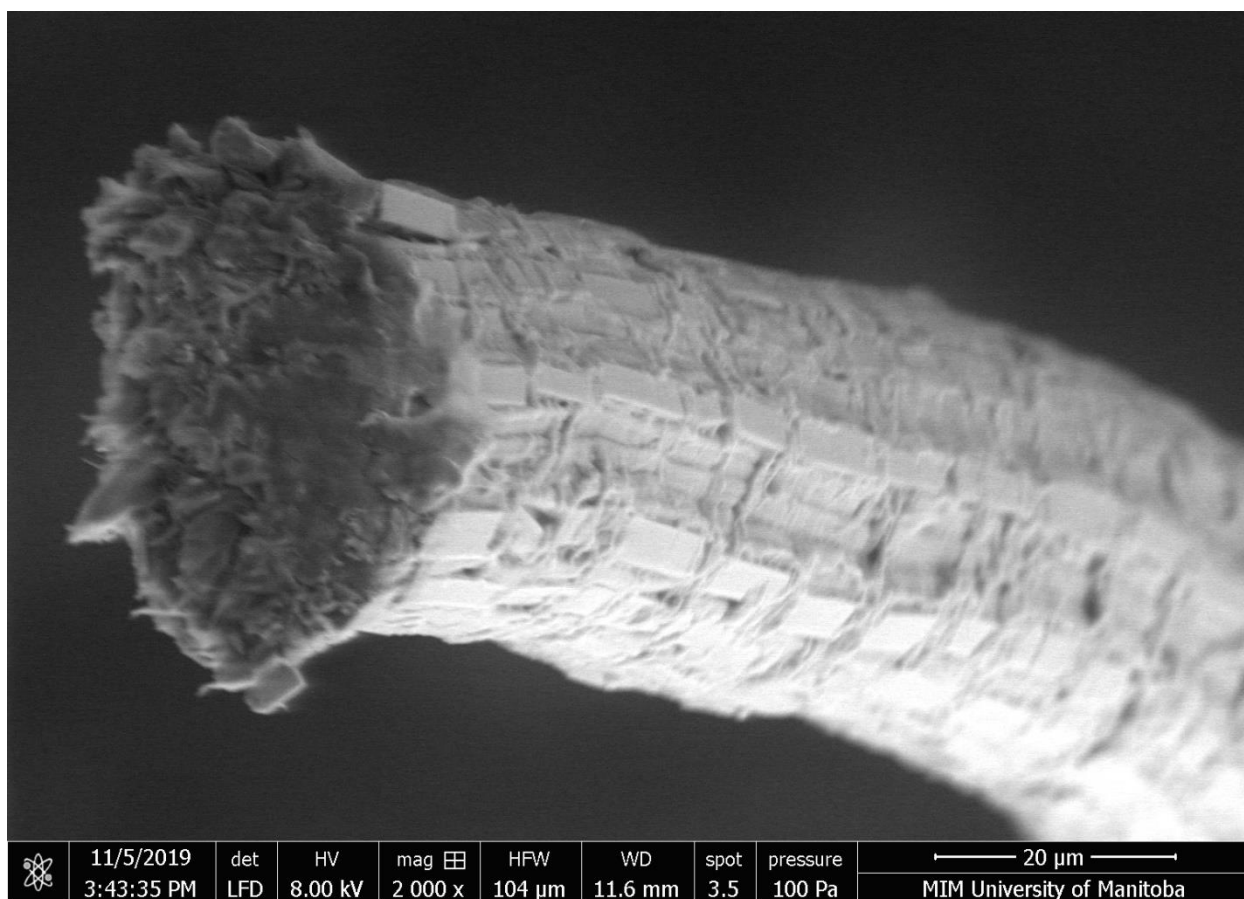


Figure 4.2.1.7. An ESEM image of a manually extracted *Typha* leaf lacunae fiber. The cross-section of it was shown.

Witztum and Wayne (2014) investigated the crystals on fiber cables isolated from a *T. x glauca* leaf and they deduced that probably these crystals were composed of calcium oxalate. Sheath cells of fiber cables of *T. domingensis* also contained calcium oxalate prismatic crystals (Witztum and Wayne 2016a). About the diaphragms, Witztum and Wayne (2016a) discussed that there were diaphragms of two or three cell layers thick, where the fibers were anchored in *T. domingensis*.

ESEM images were shown to demonstrate the morphology of the lacunae fiber and its surrounding structure. Figure 4.2.1.1 showed a manually extracted lacunae fiber. Two

morphological characteristics which were the calcium oxalate plates (Figure 4.2.1.2 and 4.2.1.3) and nodes (Figure 4.2.1.3) could be clearly observed using a higher magnification. The elemental composition of some plates on a lacunae fiber was investigated using energy-dispersive X-ray spectroscopy (EDS) and details could be referred to section 4.2.3. The morphological characteristics of nodes and calcium oxalate plates might strongly affect the value and application of this type of fiber. For example, would their presence cause adverse effects to the application? This will require further investigation in the future.

Referring to Figure 4.2.1.4, the ESEM image for a fiber anchored in a diaphragm could be observed. The node observed on the manually extracted fibers (Figure 4.2.1.1 and 4.2.1.3) might be the remainder of the diaphragm, which was not removed during the manual extraction process. Besides, Figure 4.2.1.6 and 4.2.1.7 showed the cross-sections of the lacunae fibers.

4.2.2 Diameter

The diameters of the manually extracted lacunae fibers using the method in section 4.1 were measured by using an optical microscope. The mean was 49.4 μm , the SD was 8.8 μm , the CV% ($\text{CV\%} = (\text{SD} / \text{mean}) \times 100\%$) was 17.8%, and the number of samples was 18. The diameters of nodes were not included in these data.

4.2.3 Properties of the calcium oxalate plates

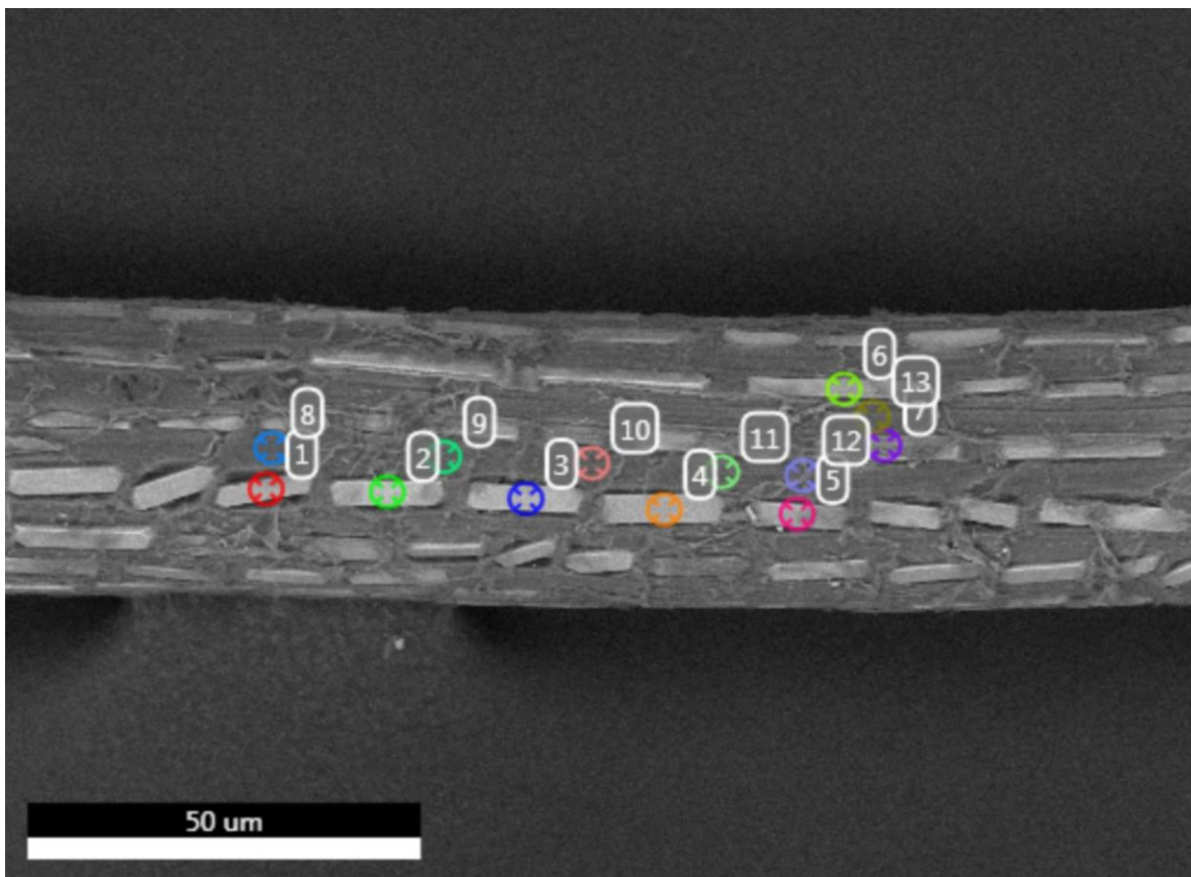


Figure 4.2.3.1. A backscattered electron image of a manually extracted lacunae fiber. Label 1 – 13 were the detection focuses (labels with different colors) of the energy-dispersive X-ray spectroscopy. Label 1 – 7 focused on the plates and label 8 – 13 focused on the non-plate places.

The elemental composition of the calcium oxalate plates was examined by using the energy-dispersive X-ray spectroscopy (EDS). The plates and non-plate places were focused (Figure 4.2.3.1). The calcium atomic % for the plates (label 1 – 7 in Figure 4.2.3.1) and non-plate places (label 8 – 13 in Figure 4.2.3.1) were calculated to be $7.64 \pm 0.51\%$ ($n = 7$) and $1.18 \pm 0.08\%$ ($n = 6$) respectively. Using Asymptotic Wilcoxon-Mann-Whitney Test, $p\text{-value} = 0.001192 < 0.05$. Therefore, the non-plate places had Ca atomic % less than that of the plates. EDS graphical result

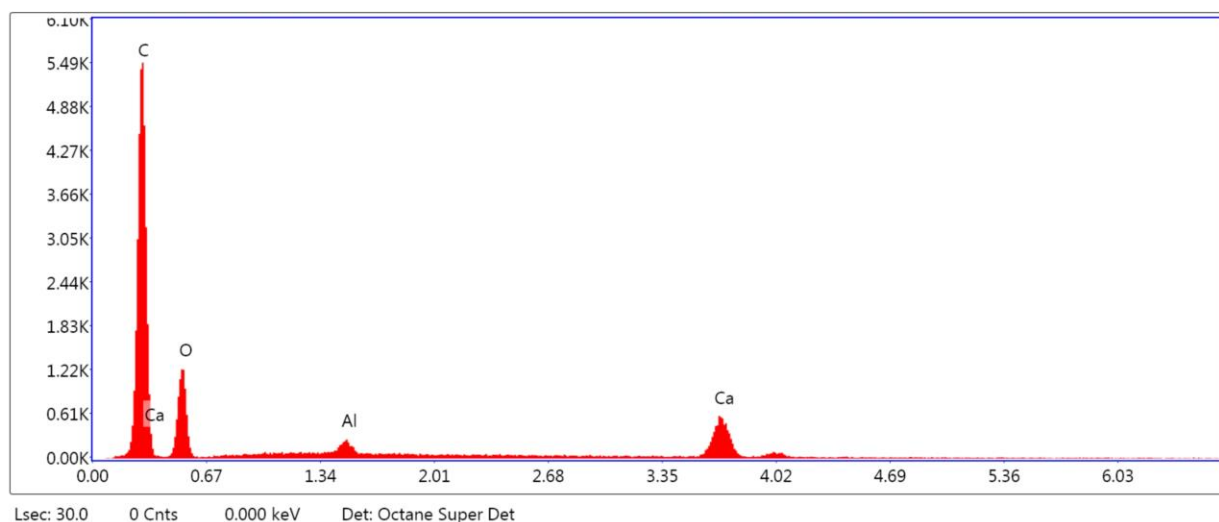


Figure 4.2.3.2. EDS results for label 1 (the plate) in Figure 4.2.3.1

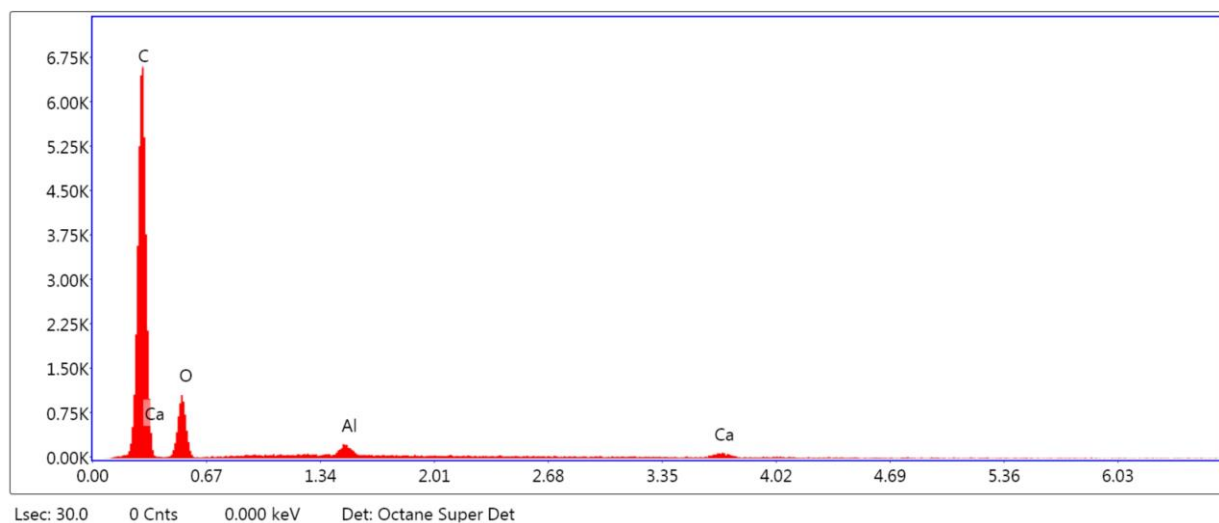


Figure 4.2.3.3. EDS results for label 8 (the non-plate place) in Figure 4.2.3.1

examples could be referred to Figure 4.2.3.2 and 4.2.3.3. The peak for Ca was higher for label 1 (plate) than label 8 (non-plate place). All the results for label 1 – 13 could be referred to Appendix II. Based on the references (Witztum and Wayne 2014, 2015, 2016a) for certain *Typha* species as well as the presence of calcium at the plates in the samples of this research, probably these plates consisted of calcium oxalate.

4.3 Botanical study on the extraction

Botanical study is crucial for explaining the extraction process and providing suggestion to design a better extraction method. For *Typha latifolia* L., the inner structure of lamina and sheath were constructed with continuous columns of lacunae. These columns were separated by partitions and supported by fibrous strands in the longitudinal direction and horizontal diaphragms. The continuous internal structure involved only gradual changes between the sheath and lamina. The stellate cell wisps were abundant in the sheath, the amount became fewer gradually in the lamina, and they were absent in the distal lamina (Rowlatt and Morshead 1992). In Figure 4.2.1.4, it could be observed that the lacunae fiber was anchored in the diaphragm, which was found next to the partition. The non-diaphragm aerenchyma with foam-like structure (Figure 4.2.1.5 and 4.2.1.6) enclosed the lacunae fiber (Figure 4.2.1.6).

The rationale to extract the lacunae fibers manually was to selectively remove the surrounding structural materials. The aerenchyma held the lacunae fibers in place and removing the aerenchyma could isolate the lacunae fibers. The experimental experience in this research for the manual extraction process was that there was less hindrance in the leaf sheath with more non-diaphragm aerenchyma; the non-diaphragm aerenchyma and diaphragms (except the nodes on the fibers) were components which could be removed easily; the manual extraction process was more difficult in the lamina with less non-diaphragm aerenchyma as removing the dorsal surface became more difficult in the lamina.

Although the *Typha* species was not an investigation focus in this research, understanding the properties of the lacunae fiber cables in different species is important because not all types of *Typha* plants have this kind of fiber cable in their leaves. For example, *T. angustifolia*, *T. latifolia*, *T. × glauca*, *T. domingensis*, and *T. shuttleworthii* had lacunae fiber cables; *T. minima* and *T.*

elephantina did not have lacunae fiber cables; lacunae fiber cables of *T. laxmannii* had only a few centimeters of extension from the leaf sheath into the leaf blade; for the leaf blade of *T. domingensis*, the lacunae fiber cables were absent from the very distal part (Witztum and Wayne 2014, 2016a).

CHAPTER 5. Treatment of the manually extracted lacunae fibers

5.1 Treatment with an alkaline chemical at room temperature

The manual extraction method discussed in chapter 4 showed that the knowledge from the botanical study could be applied in the fiber engineering area. It is too early to suggest whether the lacunae fibers obtained from the manual extraction could replace the chemically extracted fibers because of two reasons: Firstly, the type of fiber extracted in the chemical extraction was not clear (this would be further explained in section 7.2) and secondly, the research on the fiber properties and applications has not reached industrial level.

Moreover, a very important point was that chemical treatment was still applicable for the manually extracted fibers. This idea is worth the consideration because it was not proposed that the manual extraction method in this thesis could avoid all kinds of chemical treatments of fibers. The use of an alkaline solution in the chemical extraction of cattail leaf fibers had the extraction purpose to remove the structural materials although the alkaline chemical could have other effects, e.g. changing the crystalline property (Mwaikambo and Ansell 2002). But for the manual extraction, the alkaline chemical would be expected mainly for fiber engineering purposes other than extraction (this idea also depends on how a fiber is considered as extracted). Apart from that, would the manually extracted fibers after the alkaline treatment be the same (or similar in the aspects of botany, morphology, and diameter) as the chemically extracted fibers? In this chapter, potassium hydroxide solution was examined for its effect towards the manually extracted lacunae fiber, while the comparison of its properties with the untreated lacunae fibers and chemically extracted fibers would be analyzed and discussed in chapter 7.

5.2 Characterization of the fibers with alkaline treatment at room temperature

The manually extracted *Typha* leaf lacunae fibers were treated with 5 wt% potassium hydroxide solution for 2 hours at room temperature. After that, water was used to wash the treated fibers. The 5 wt% potassium hydroxide solution was prepared by dissolving the potassium hydroxide pellet in water. 5 wt% was calculated by assuming the density of water to be 1 g/mL. $\text{wt\%} = (\text{mass of KOH} / \text{mass of water}) \times 100\%$. Note that the water density assumption and wt% calculation were also applied to the other experiments which involved the potassium hydroxide solution preparation in this research.

5.2.1 Morphology

Figure 5.2.1.1 showed a lacunae fiber obtained from the manual extraction method and treated with 5 wt% KOH solution for 2 hours at room temperature. Figure 5.2.1.2 showed a chemically treated (at room temperature) manually extracted lacunae fiber with a higher magnification. Figure 5.2.1.3 showed a chemically treated (at room temperature) manually extracted lacunae fiber with a higher magnification. Figure 5.2.1.1 – 5.2.1.3 were ESEM images. Figure 5.2.1.4 showed a chemically treated (at room temperature) manually extracted lacunae fiber. Figure 5.2.1.5 showed a chemically treated (at room temperature) manually extracted lacunae fiber with a higher magnification. Figure 5.2.1.4 and 5.2.1.5 were backscattered electron images. Based on these images, there were nodes and calcium oxalate plates on the lacunae fiber after the chemical treatment at room temperature.

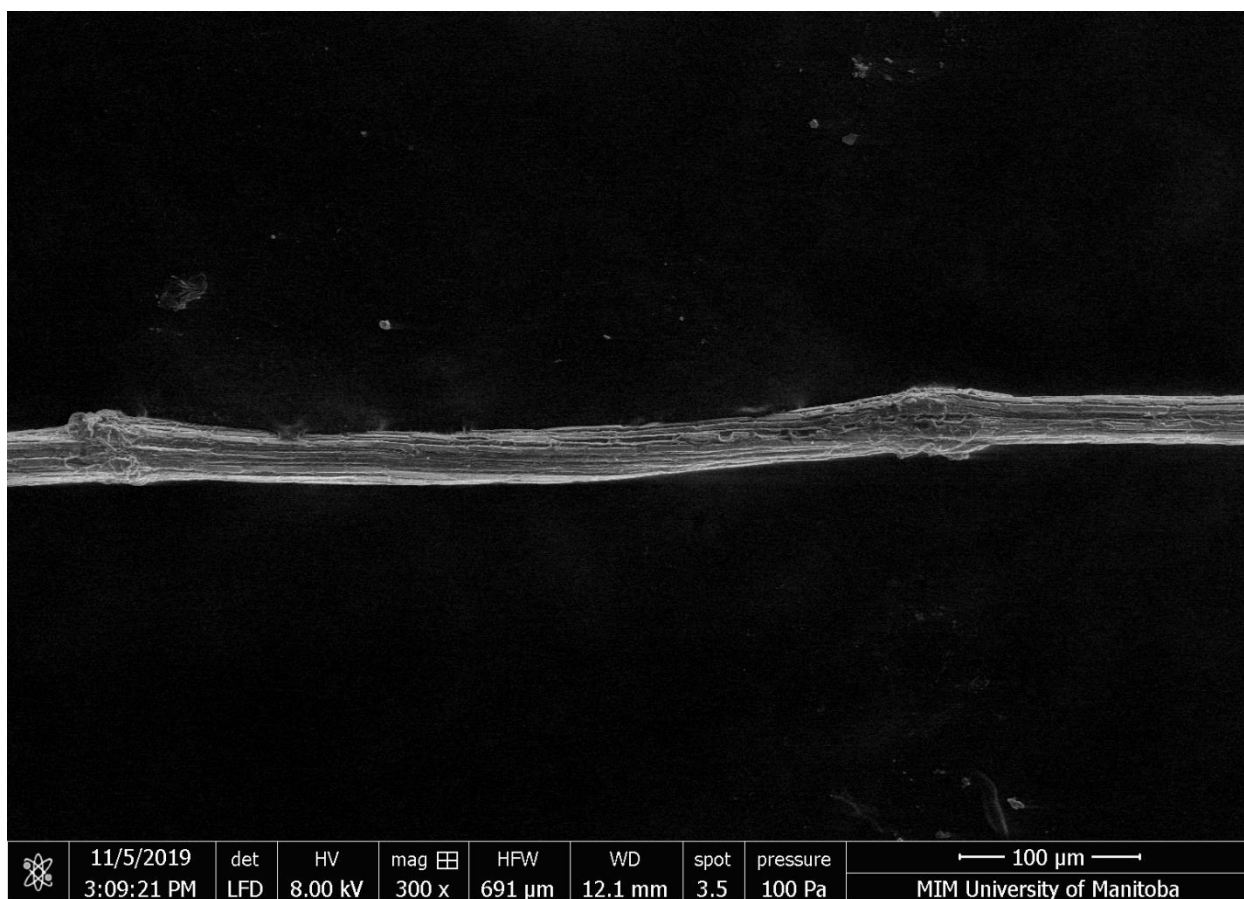


Figure 5.2.1.1. An ESEM image of a lacunae fiber obtained from the manual extraction method and treated with 5 wt% KOH solution for 2 hours at room temperature. Nodes and calcium oxalate plates could be observed.

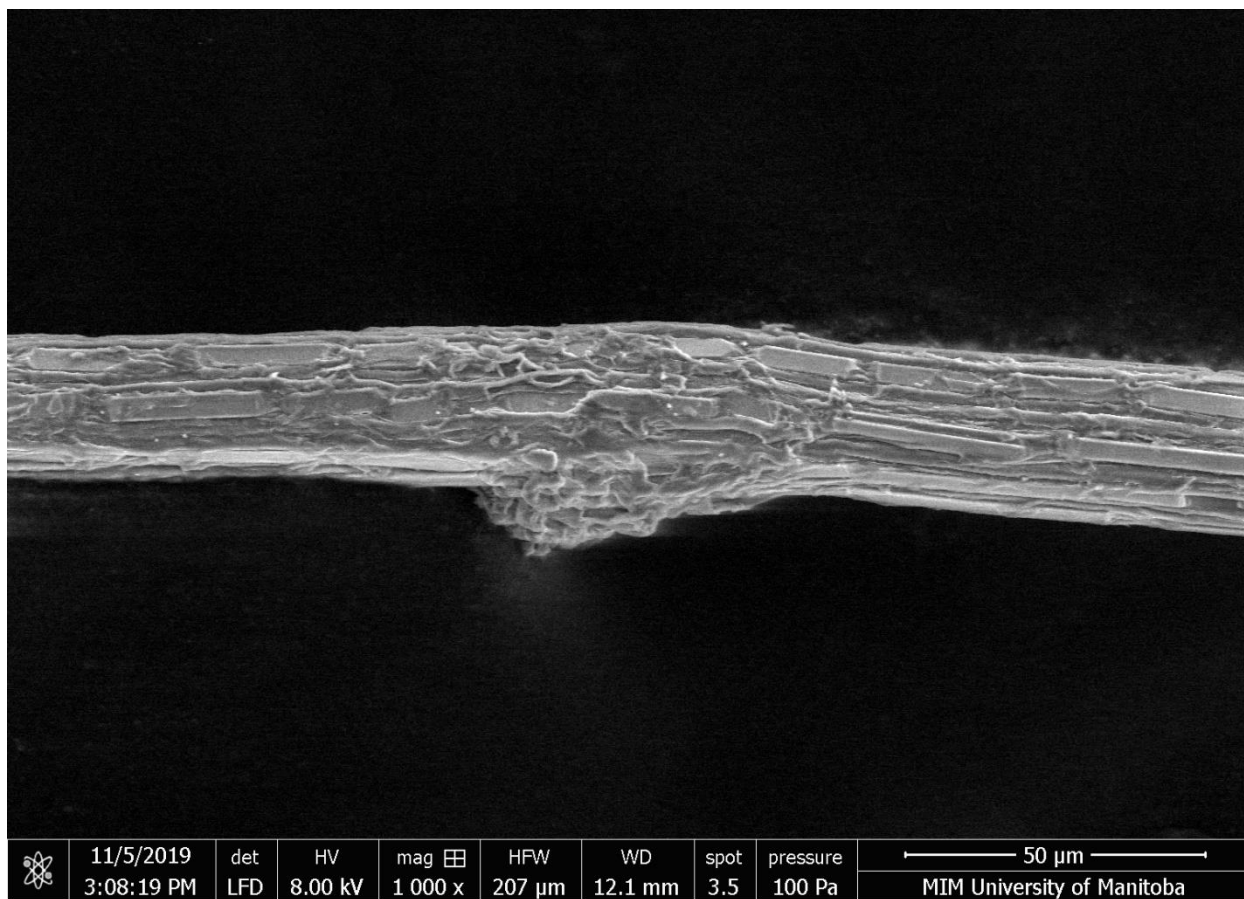


Figure 5.2.1.2. An ESEM image of a closer view of a chemically treated (at room temperature) manually extracted lacunae fiber. A node and calcium oxalate plates could be observed.

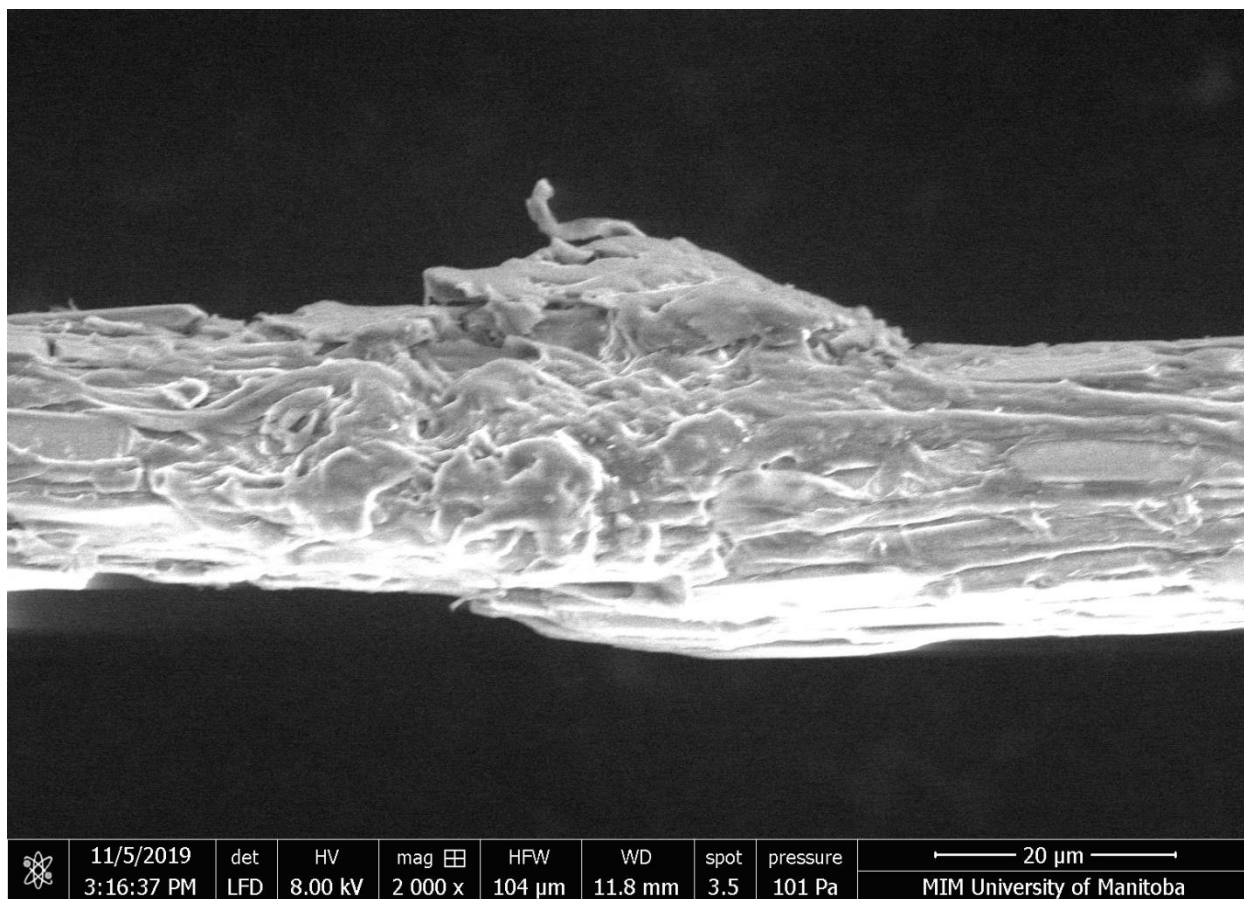


Figure 5.2.1.3. An ESEM image of a closer view of a chemically treated (at room temperature) manually extracted lacunae fiber with a higher magnification. A node and calcium oxalate plates could be observed.

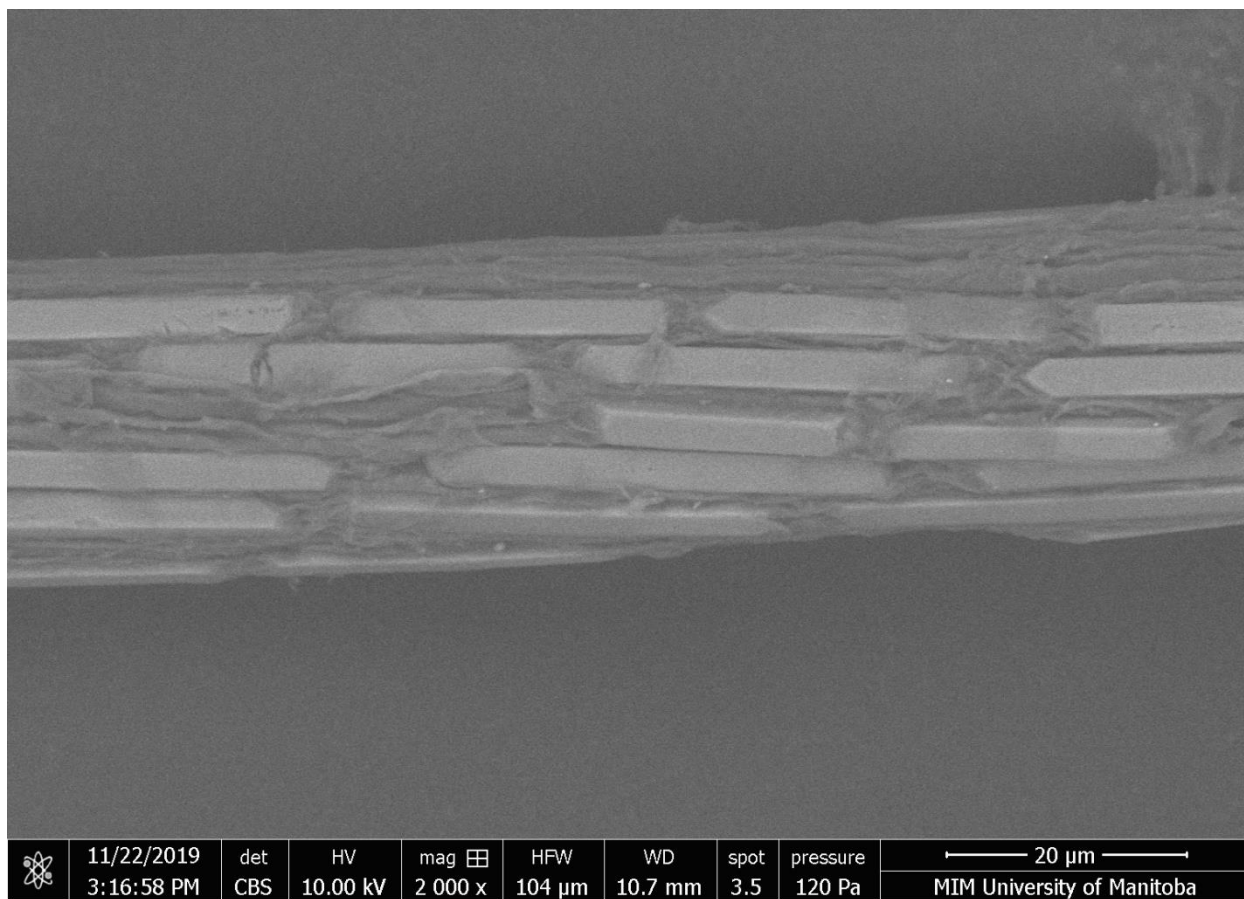


Figure 5.2.1.4. A backscattered electron image of a chemically treated (at room temperature) manually extracted lacunae fiber. Calcium oxalate plates could be observed.

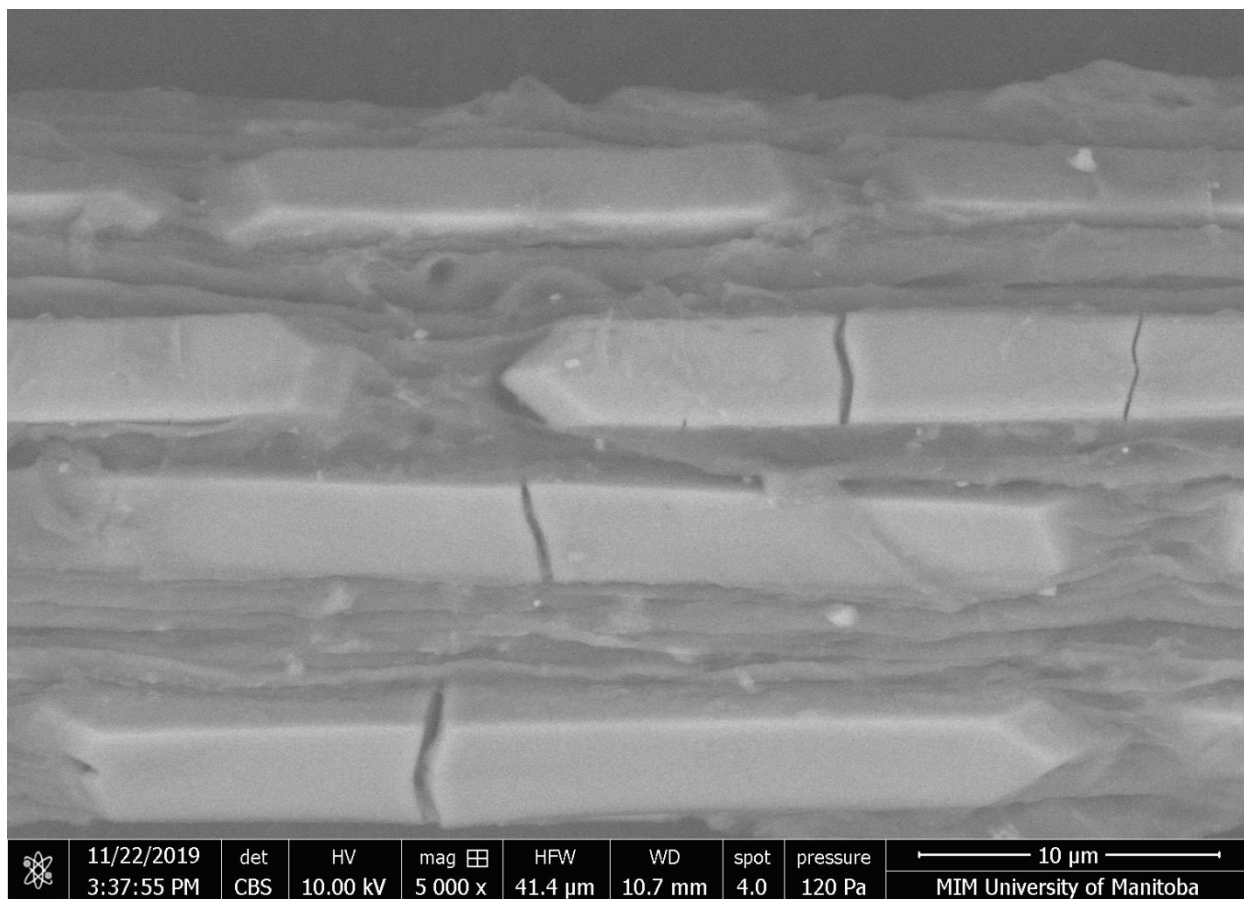


Figure 5.2.1.5. A backscattered electron image of a closer view of a chemically treated (at room temperature) manually extracted lacunae fiber. Calcium oxalate plates could be observed.

5.2.2 Diameter

The diameters of the manually extracted lacunae fibers which were treated with 5 wt% KOH solution for 2 hours at room temperature were measured by using an optical microscope. The mean was 30.6 μm ; the SD was 2.1 μm ; the CV% was 6.8%; the number of samples was 18. The diameters of the nodes were not included in these data.

5.3 Treatment with an alkaline chemical at higher temperature

From the experimental results of the chemical treatment of manually extracted lacunae fibers at room temperature, it could be observed that the nodes and calcium oxalate plates were still present. The effect of temperature was investigated for the surface morphology and diameter. The conditions were similar to the samples (chemical treatment of manually extracted lacunae fibers at room temperature) in section 5.1 and 5.2, except that higher temperature with a range of 70 – 80°C and a lower sample to alkaline solution mass ratio (the sample had a lower mass percentage) were used for the chemical treatment, but the sample to solution mass ratio was not an investigation focus in this thesis.

5.4 Characterization of the fibers with alkaline treatment at higher temperature

The manually extracted *Typha* leaf lacunae fibers were treated with 5 wt% potassium hydroxide solution, which was prepared by dissolving the potassium hydroxide pellet in water. The sample to alkaline solution mass ratio was lower in this experiment than that at room temperature. The lacunae fibers were treated with the alkaline solution for 2 hours at 70 – 80°C. After that, water was used to wash the treated fibers.

5.4.1 Morphology

Figure 5.4.1.1 showed a lacunae fiber obtained from the manual extraction method and treated with 5 wt% KOH solution for 2 hours at 70 – 80°C. Figure 5.4.1.2 showed a chemically treated (at 70 – 80°C) manually extracted lacunae fiber with a higher magnification. Figure 5.4.1.1 and 5.4.1.2 were ESEM images. Figure 5.4.1.3 showed a chemically treated (at 70 – 80°C) manually extracted lacunae fiber with a higher magnification. Figure 5.4.1.3 was a backscattered electron image. Based on these images, there were nodes and calcium oxalate plates on the lacunae fiber after the chemical treatment at 70 – 80°C.

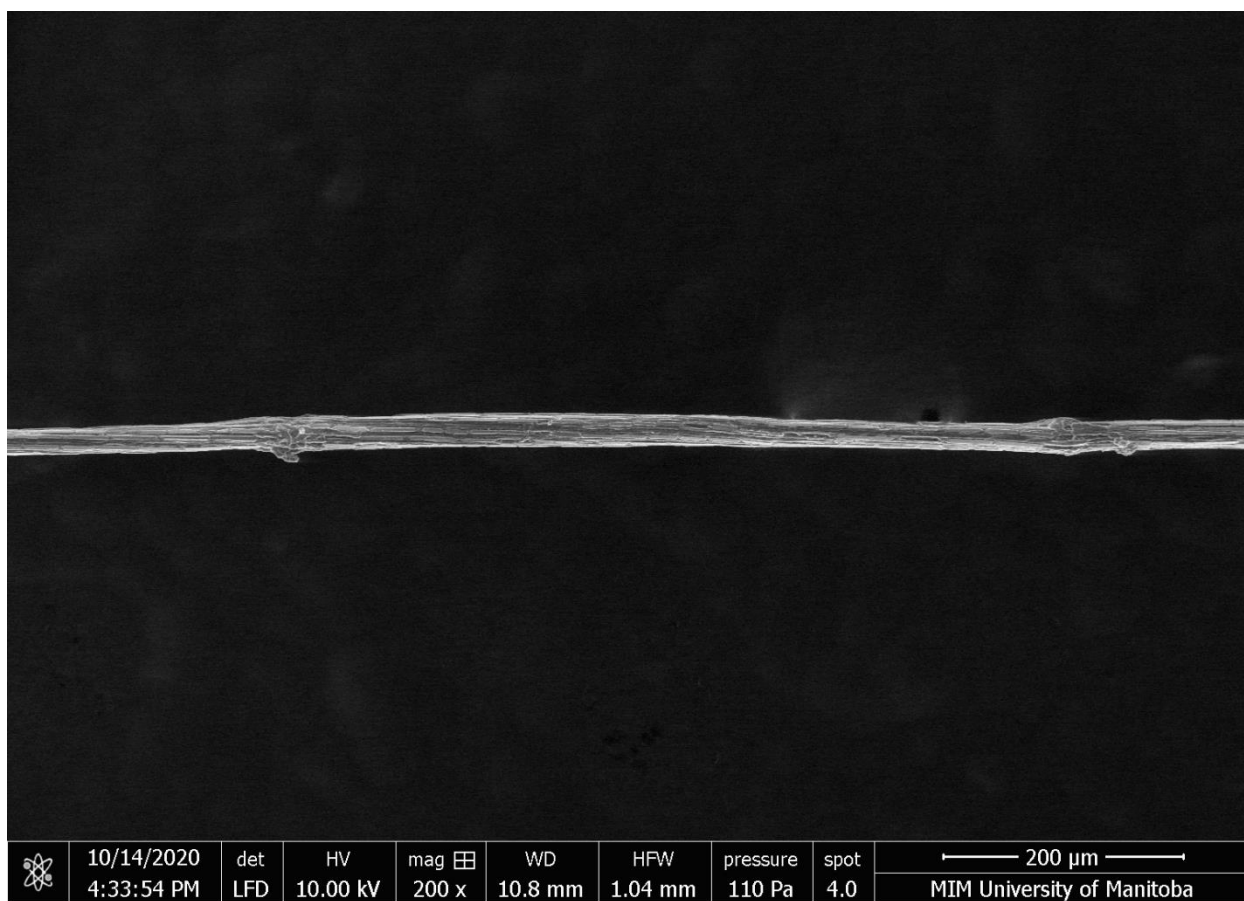


Figure 5.4.1.1. An ESEM image of a lacunae fiber obtained from the manual extraction method and treated with 5 wt% KOH solution for 2 hours at 70 – 80°C. Nodes and calcium oxalate plates could be observed.

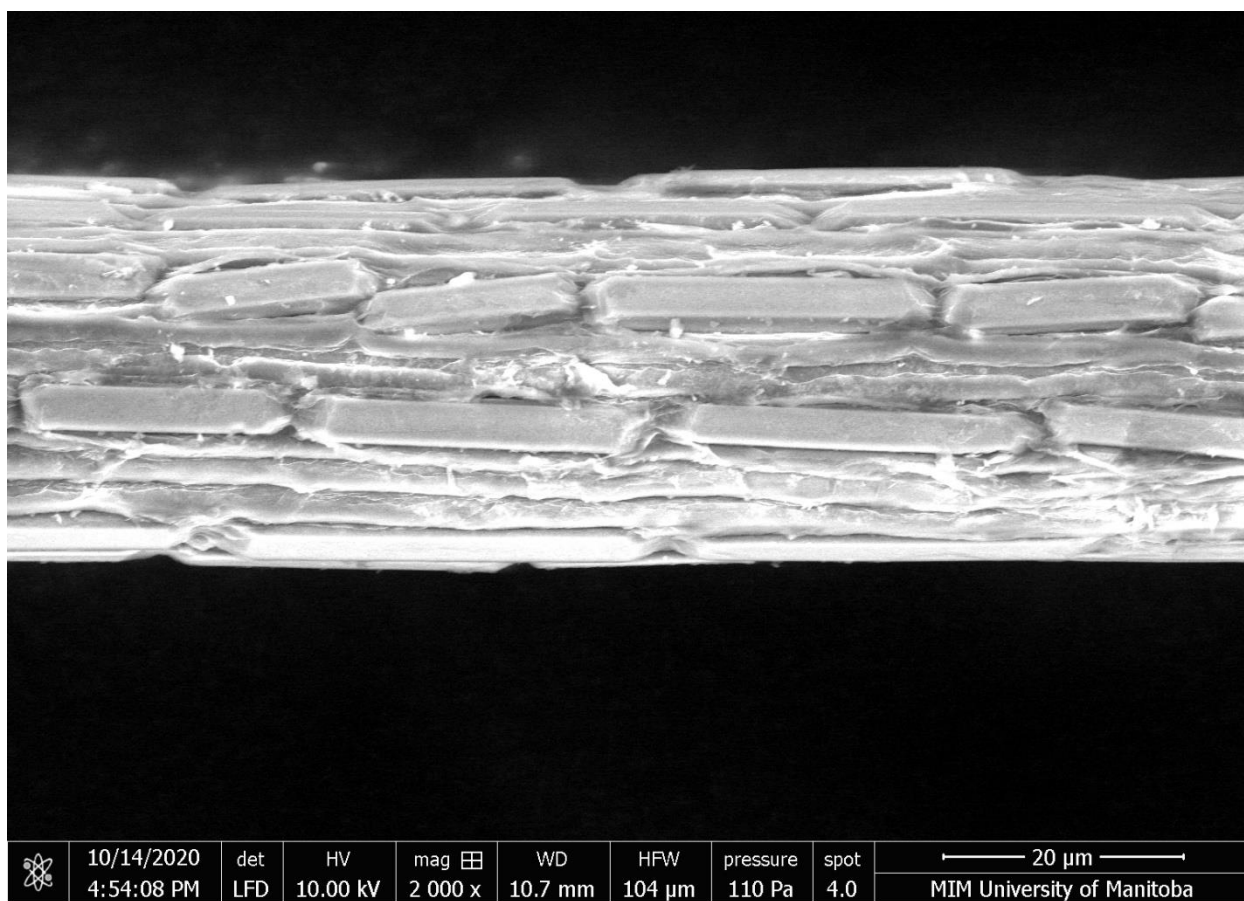


Figure 5.4.1.2. An ESEM image of a closer view of a chemically treated (at 70 – 80°C) manually extracted lacunae fiber. Calcium oxalate plates could be observed.

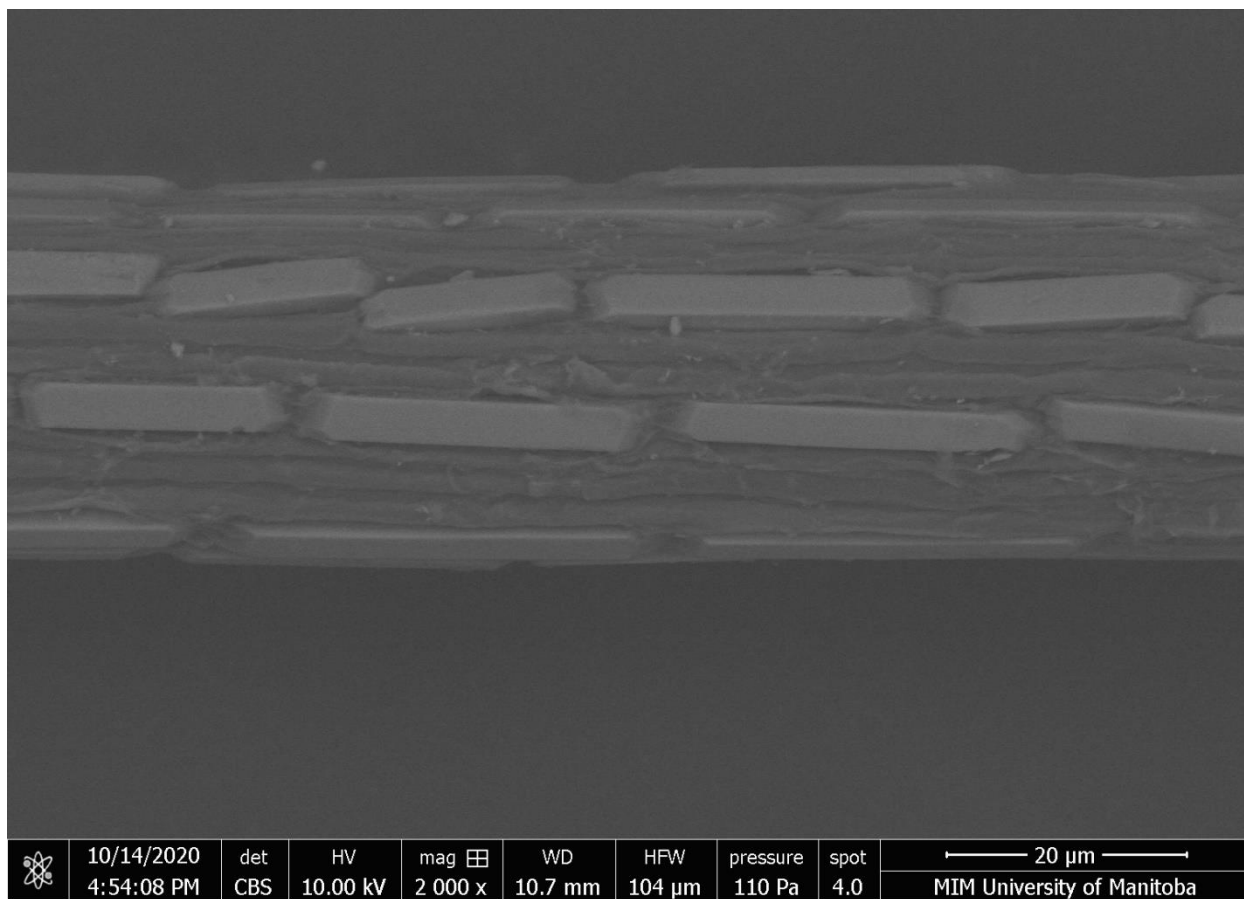


Figure 5.4.1.3. A backscattered electron image of a chemically treated (at 70 – 80°C) manually extracted lacunae fiber. Calcium oxalate plates could be observed.

5.4.2 Diameter

The diameters of the manually extracted lacunae fibers which were treated with 5 wt% KOH solution for 2 hours at 70 – 80°C were measured by using the ESEM images. The mean was 29.5 μm ; the SD was 4.8 μm ; the CV% was 16.2%; the number of samples was 14. The diameters of the nodes were not included in these data.

CHAPTER 6. Chemically extracted *Typha* leaf fibers

6.1 Chemically extracted fibers

Compared to the manual extraction method proposed in this thesis, the chemical approach involved less effort to obtain the fibers. The fiber products obtained from these two methods had difference (details would be discussed in chapter 7). The chemical method removed the outer structural materials of leaves by the chemical reaction. In section 6.2, some ESEM images of the chemically extracted fibers were demonstrated.

The conditions of the chemically extracted fibers for the ESEM imaging were that the *Typha* leaf sheaths were treated with 10 wt% potassium hydroxide solution for 2 hours at 70 – 80°C. It was also neutralized with 1 vol% of acetic acid in 30 minutes at room temperature. Note that the samples were washed by water after each process and post-extraction processes were applied to improve the quality of the fibers. For example, some (but not all) less extracted parts of the samples were cut and discarded; the fibers were separated using a glass rod so that they were less attached to each other.

6.2. Characterization of the chemically extracted fibers

6.2.1 Morphology

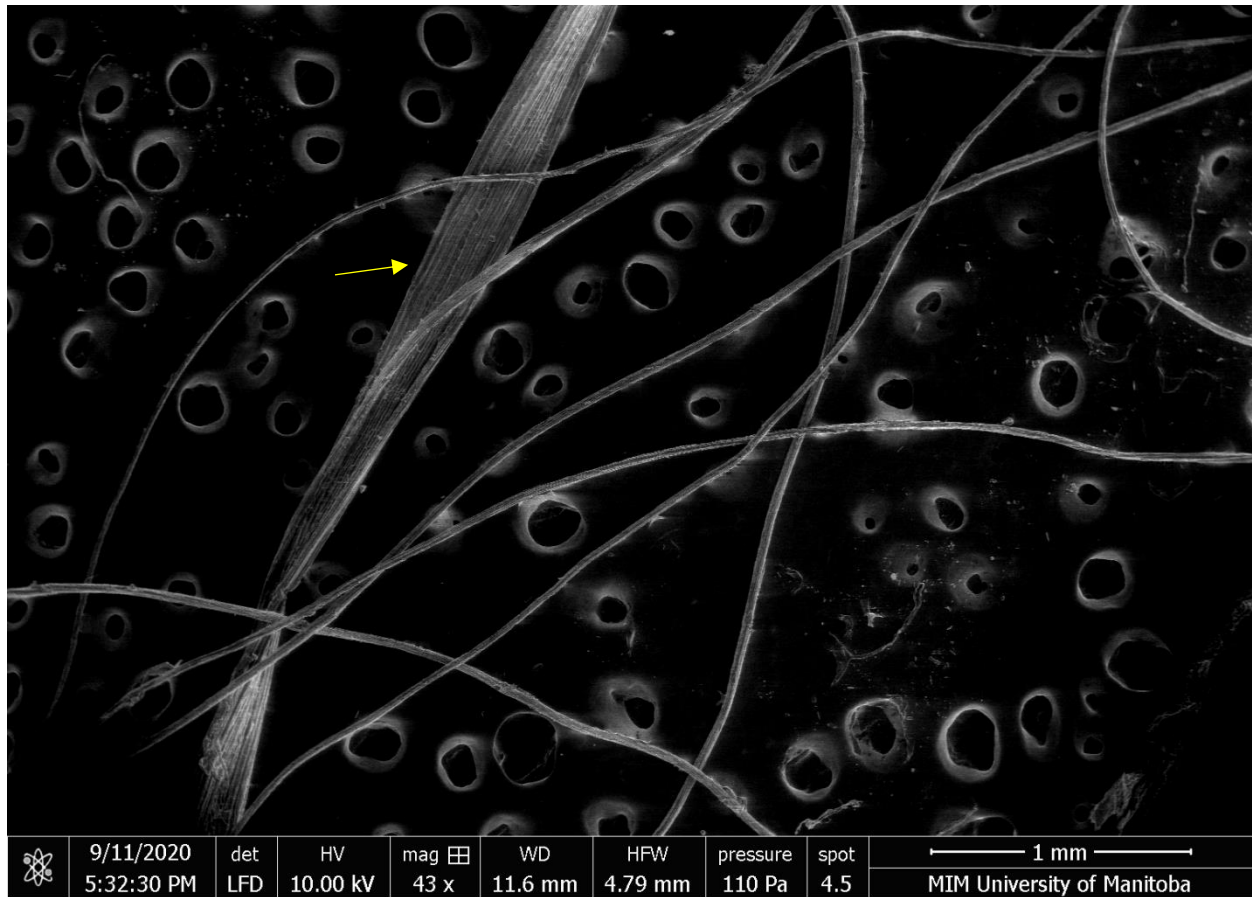


Figure 6.2.1.1. An ESEM image showing different sizes of products from the chemical extraction.

The oval shapes below should not be related to the cattail fibers. This image has shown a fiber with an observably larger diameter than the other thinner fibers (indicated by the yellow arrow).

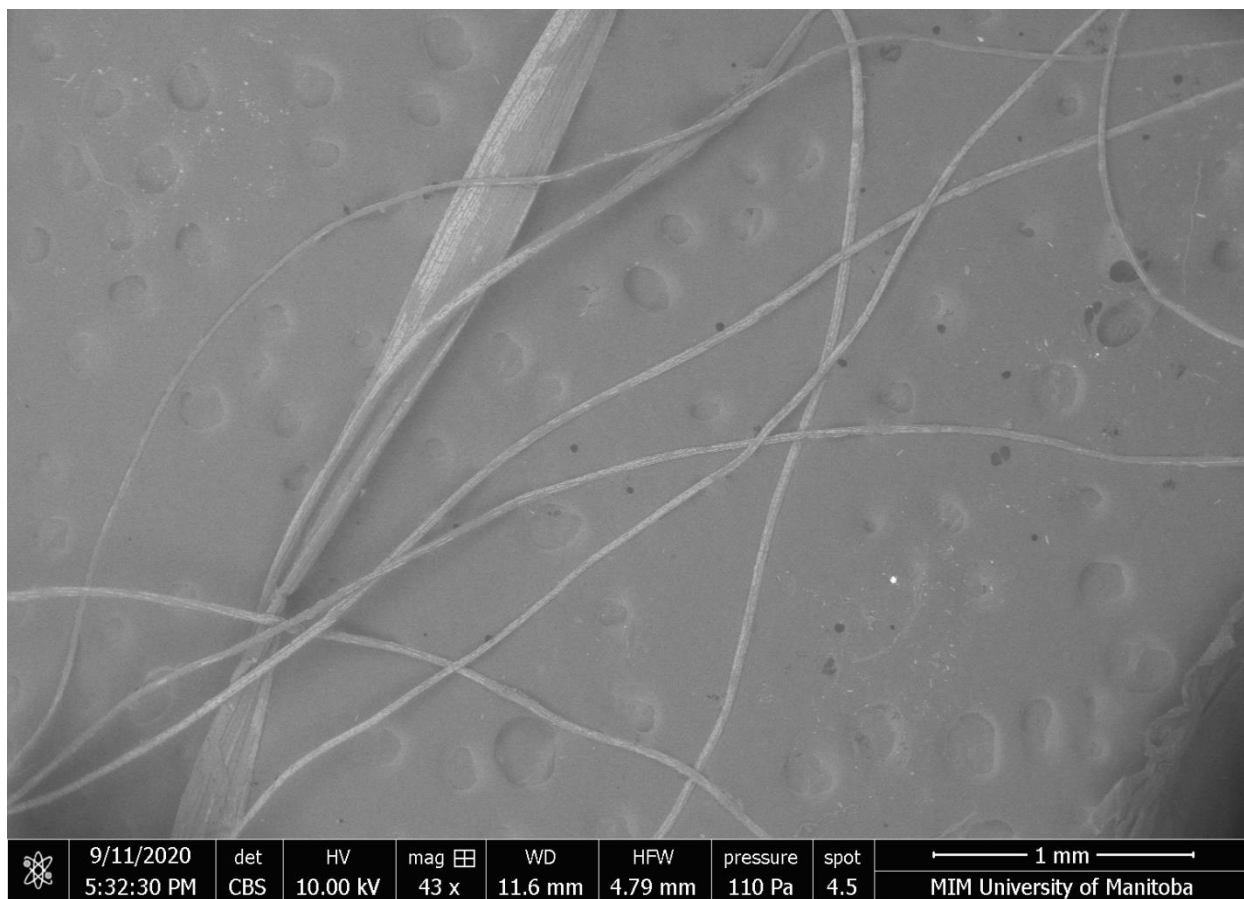


Figure 6.2.1.2. A backscattered electron image capturing the same place as Figure 6.2.1.1. Calcium oxalate plates appeared to have a whiter color in this mode. All (or most) of the fibers had calcium oxalate plates on their surfaces in this image.

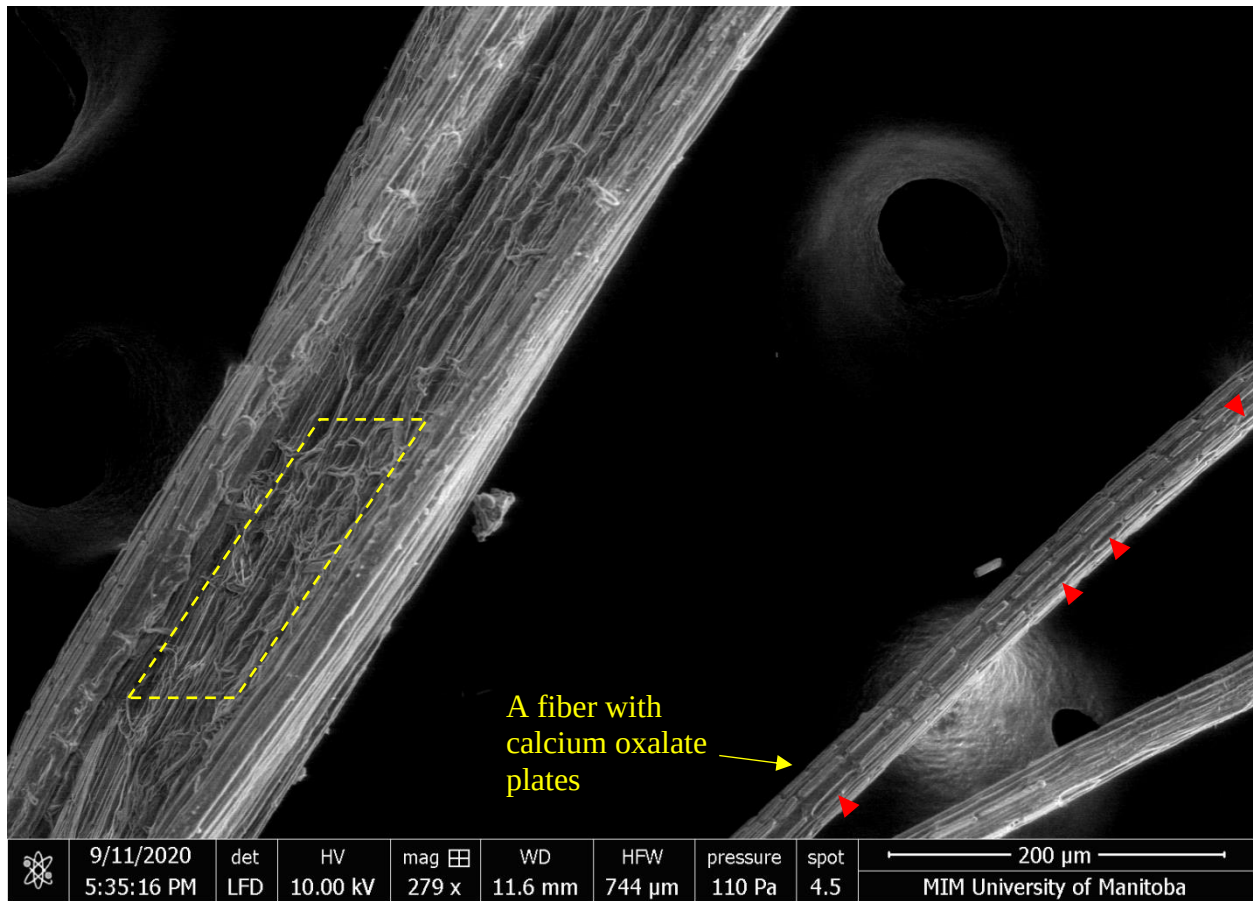


Figure 6.2.1.3. This ESEM image had a larger magnification to zoom in on the fiber with a relatively larger diameter in Figure 6.2.1.1. It could be observed that there were potentially some unremoved aerenchyma (an example was indicated by the area with yellow dashed sides). Apart from that, for the fiber with calcium oxalate plates (indicated by the yellow arrow), if only a column of calcium oxalate plates was focused, four pits could be observed (indicated by the four red arrowheads). They were deduced to be the surface morphology of the lacunae fiber after the calcium oxalate plates were removed from these positions.

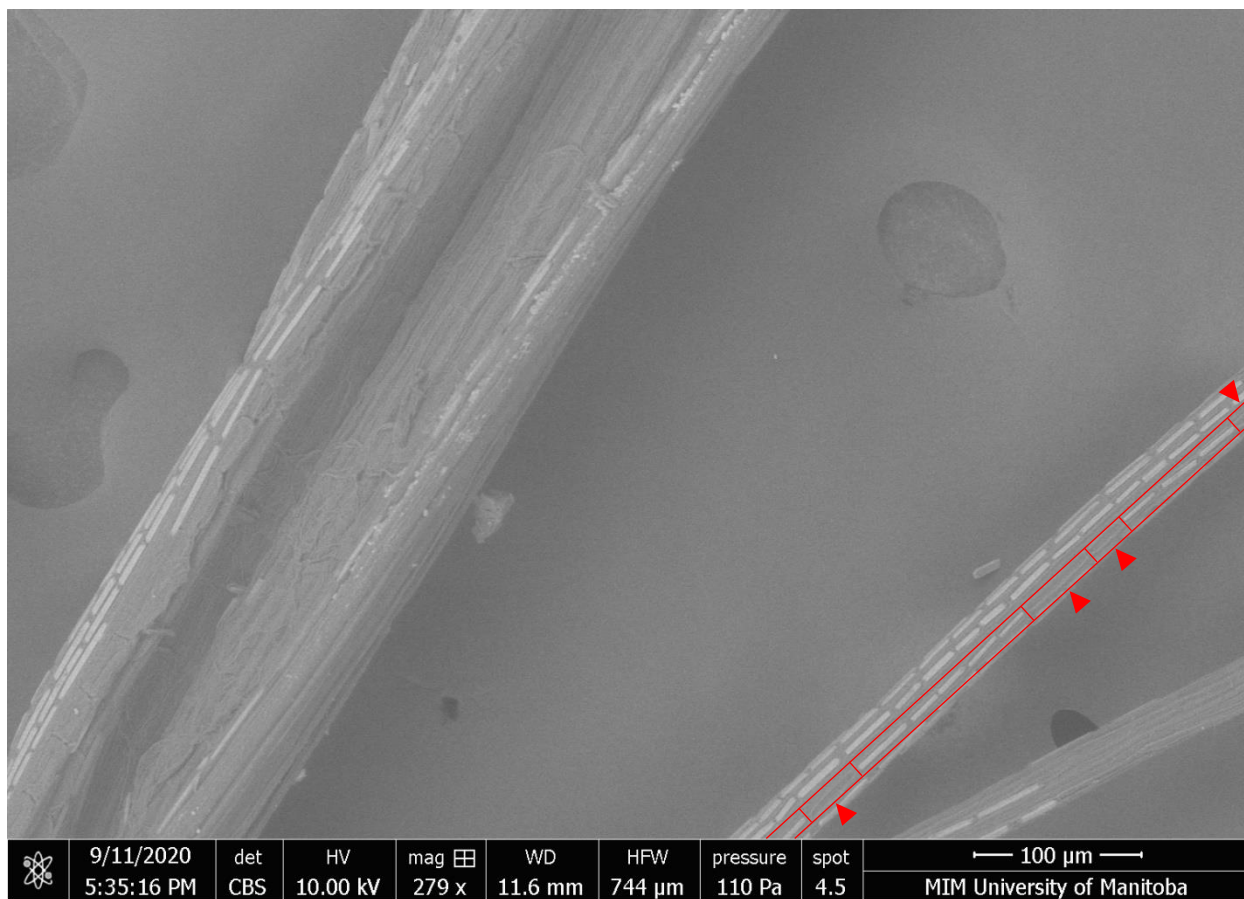


Figure 6.2.1.4. A backscattered electron image capturing the same place as Figure 6.2.1.3. Calcium oxalate plates appeared to have a whiter color in this mode. The column of calcium oxalate plates and places which had the calcium oxalate plates removed were indicated by the red lines and arrowheads.

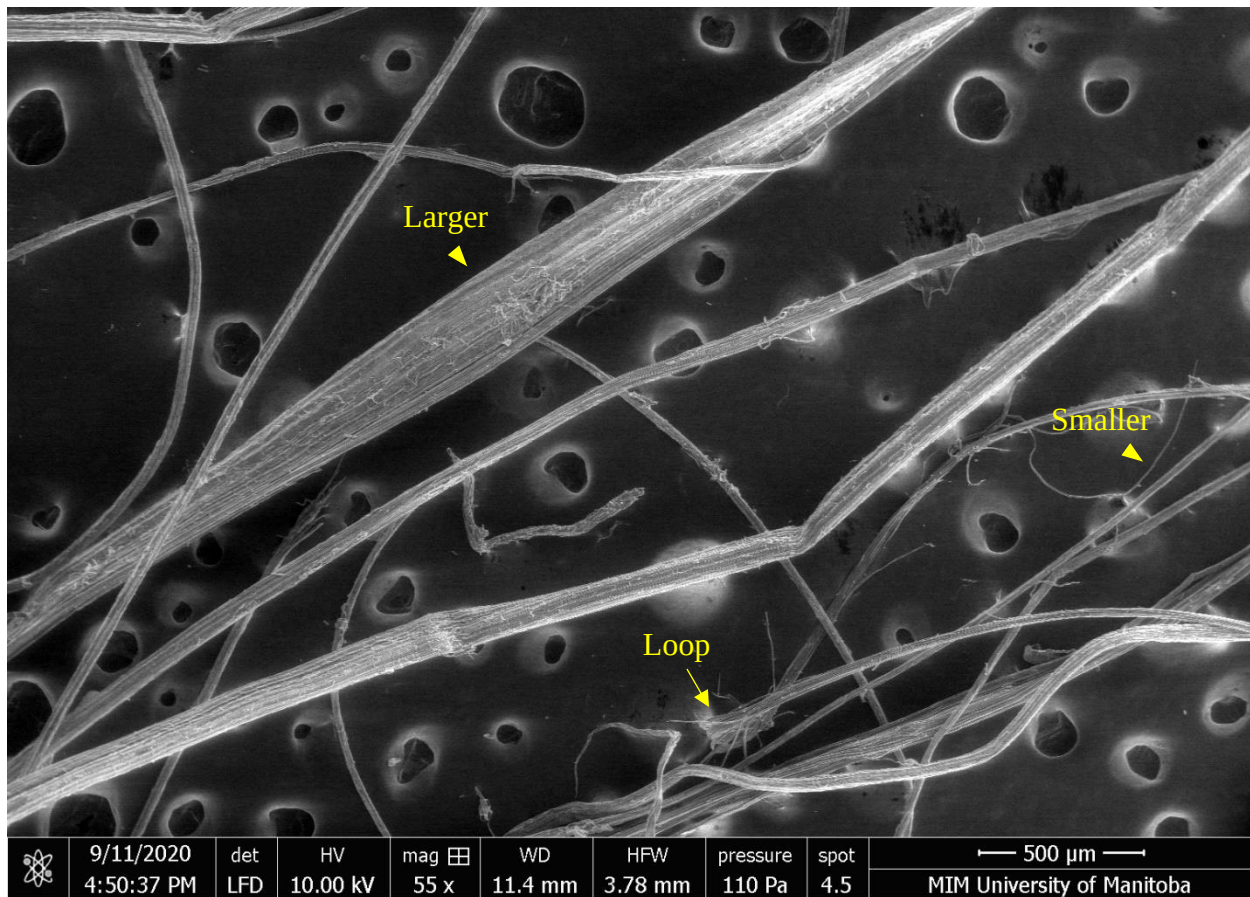


Figure 6.2.1.5. An ESEM image showing different sizes of products from the chemical extraction. The oval shapes below should not be related to the cattail fibers. This image captured fibers with a large variation in diameter. For example, the two yellow arrowheads indicated the two fibers with an observably larger and smaller diameter. There was a fiber or fiber bundle (depends on the definition of a fiber bundle) which turned around at a position and formed a loop (indicated by the yellow arrow). Its thinner structural fibers had been separated and the ESEM image with a higher magnification on this part could be referred to Figure 6.2.1.7.



Figure 6.2.1.6. A backscattered electron image capturing the same place as Figure 6.2.1.5. Some fibers had calcium oxalate plates on their surfaces in this image.

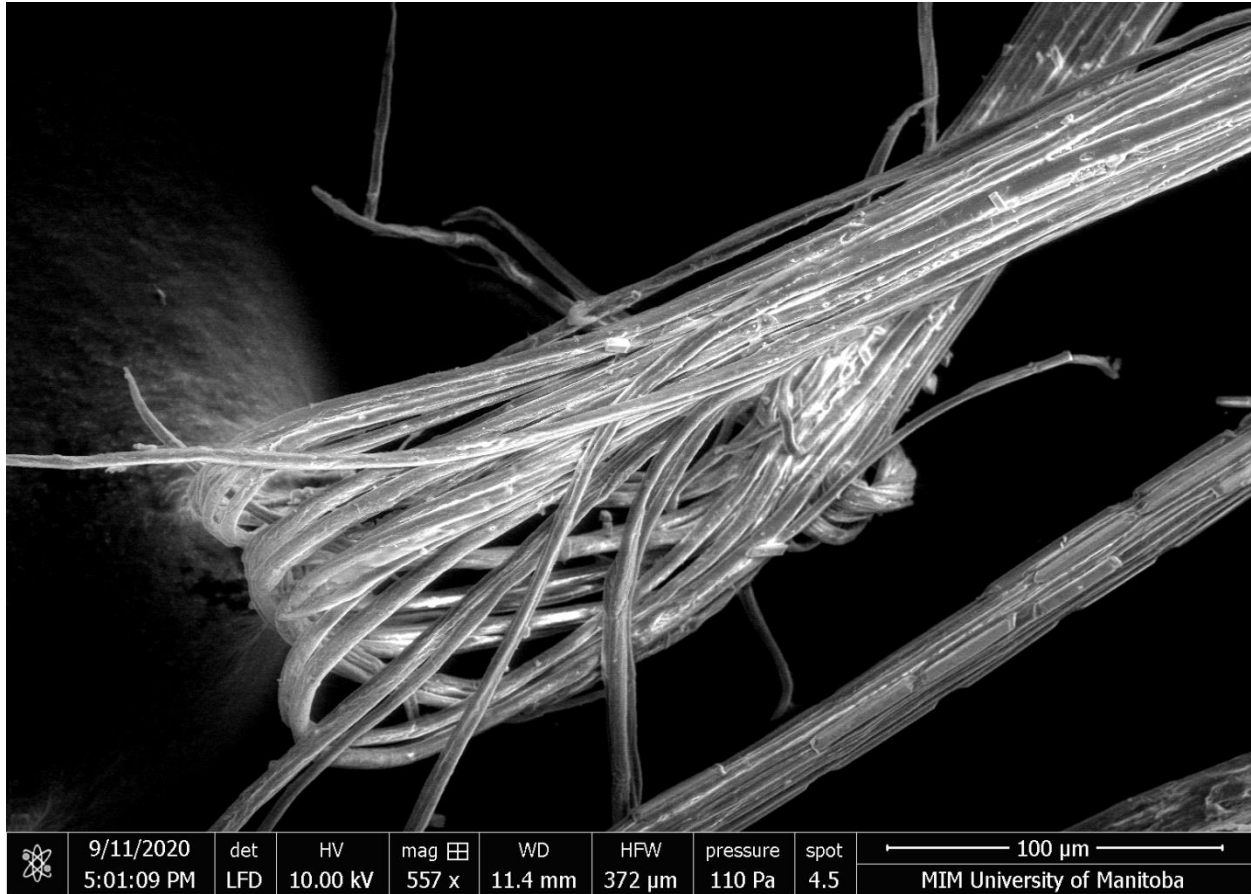


Figure 6.2.1.7. This ESEM image had a larger magnification to zoom in on the loop position in Figure 6.2.1.5. It could be observed that the structural fibers were separated at this loop position with both ends being bundles. The reason of having a loop was not the focus here, but the structural fibers with smaller diameters found in this loop showing its structural dissociation were.

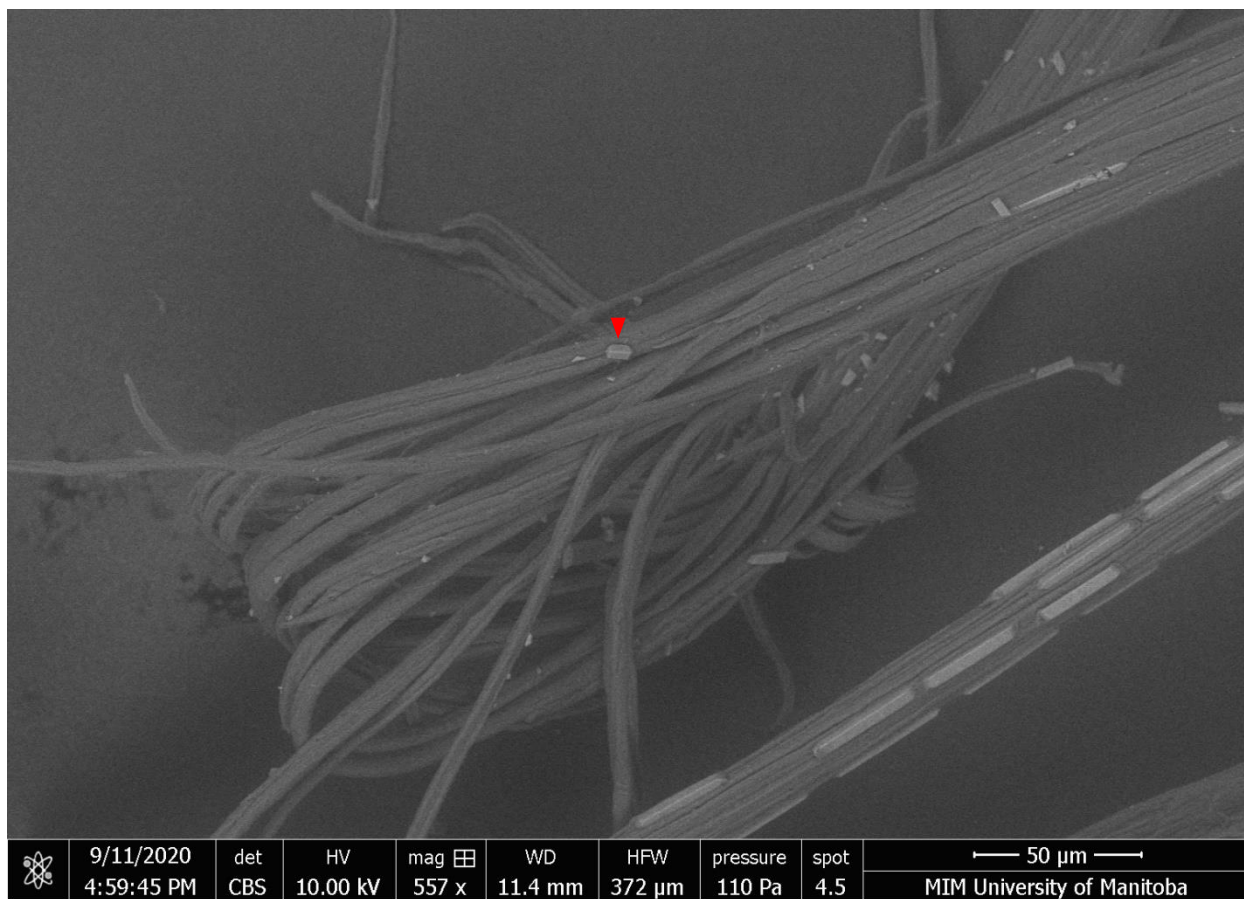


Figure 6.2.1.8. A backscattered electron image capturing the same place as Figure 6.2.1.7. The whiter substances should be the calcium oxalate materials. There was calcium oxalate debris on the fiber loop. An example was indicated by the red arrowhead.

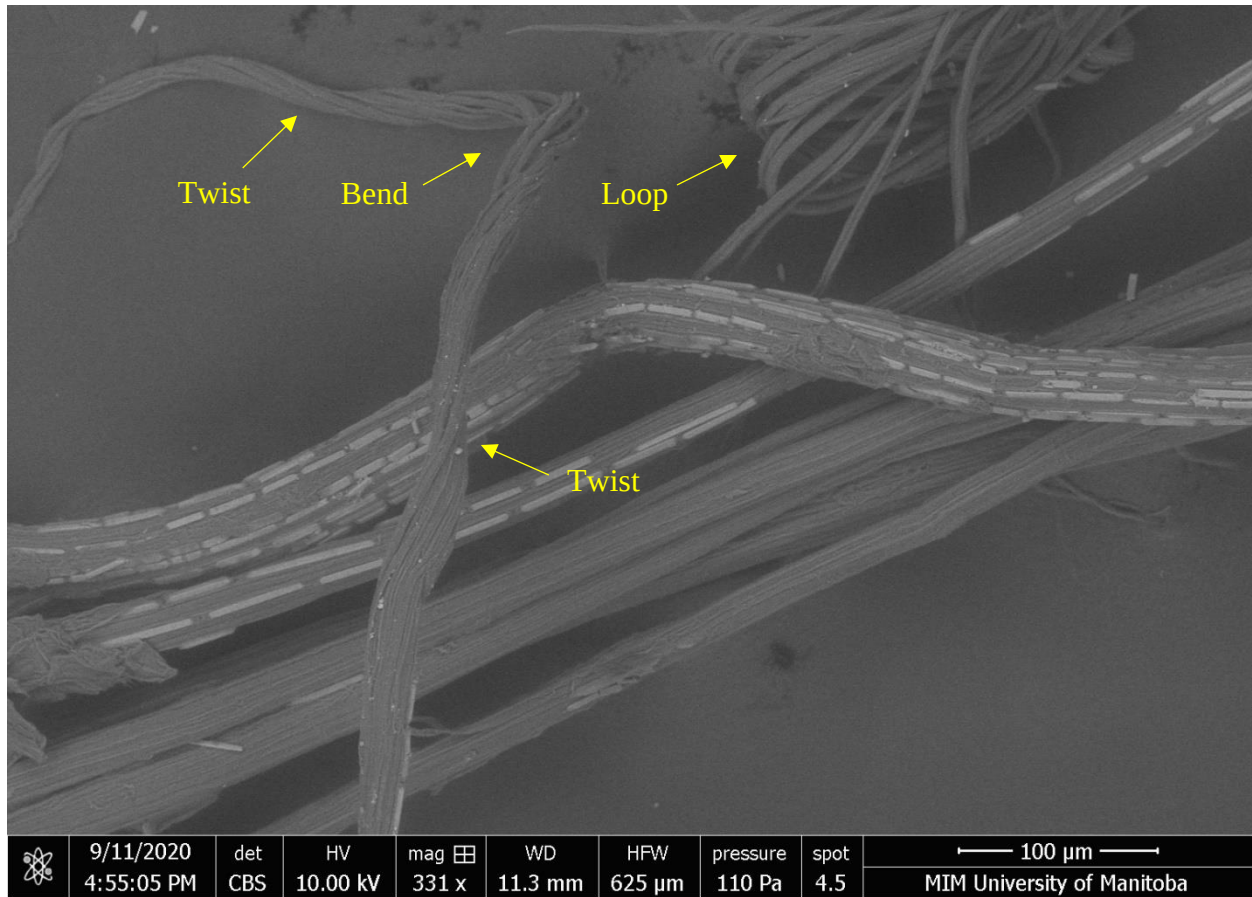


Figure 6.2.1.9. A backscattered electron image capturing a twisted fiber (or fiber bundle) near the loop in Figure 6.2.1.7 and 6.2.1.8. The reason of having a bend was not the focus here, but the structural fibers with smaller diameters found in this bend showing its structural dissociation were.

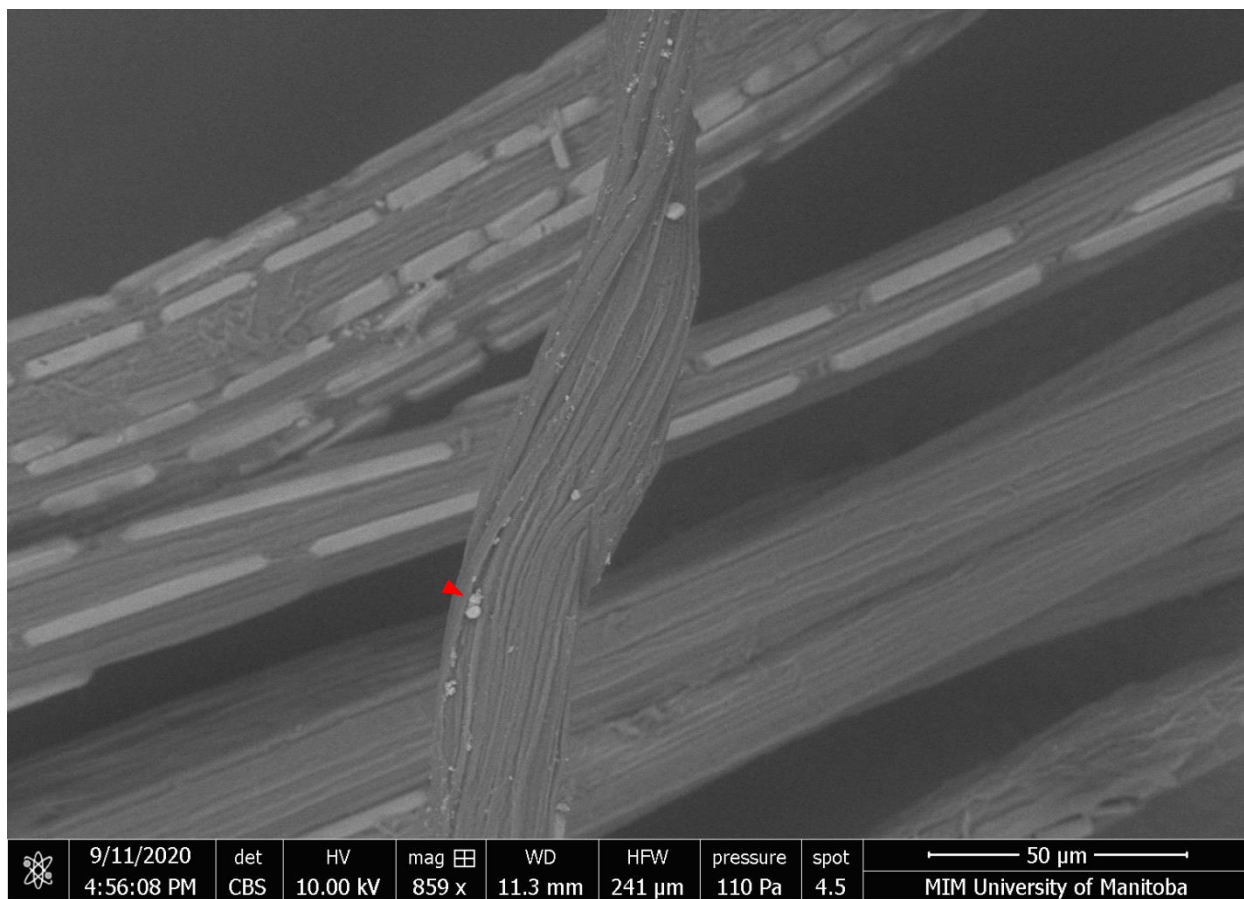


Figure 6.2.1.10. A backscattered electron image with a larger magnification zoomed in on Figure 6.2.1.9. Calcium oxalate debris could be observed on the fiber (or fiber bundle). An example was indicated by the red arrowhead.

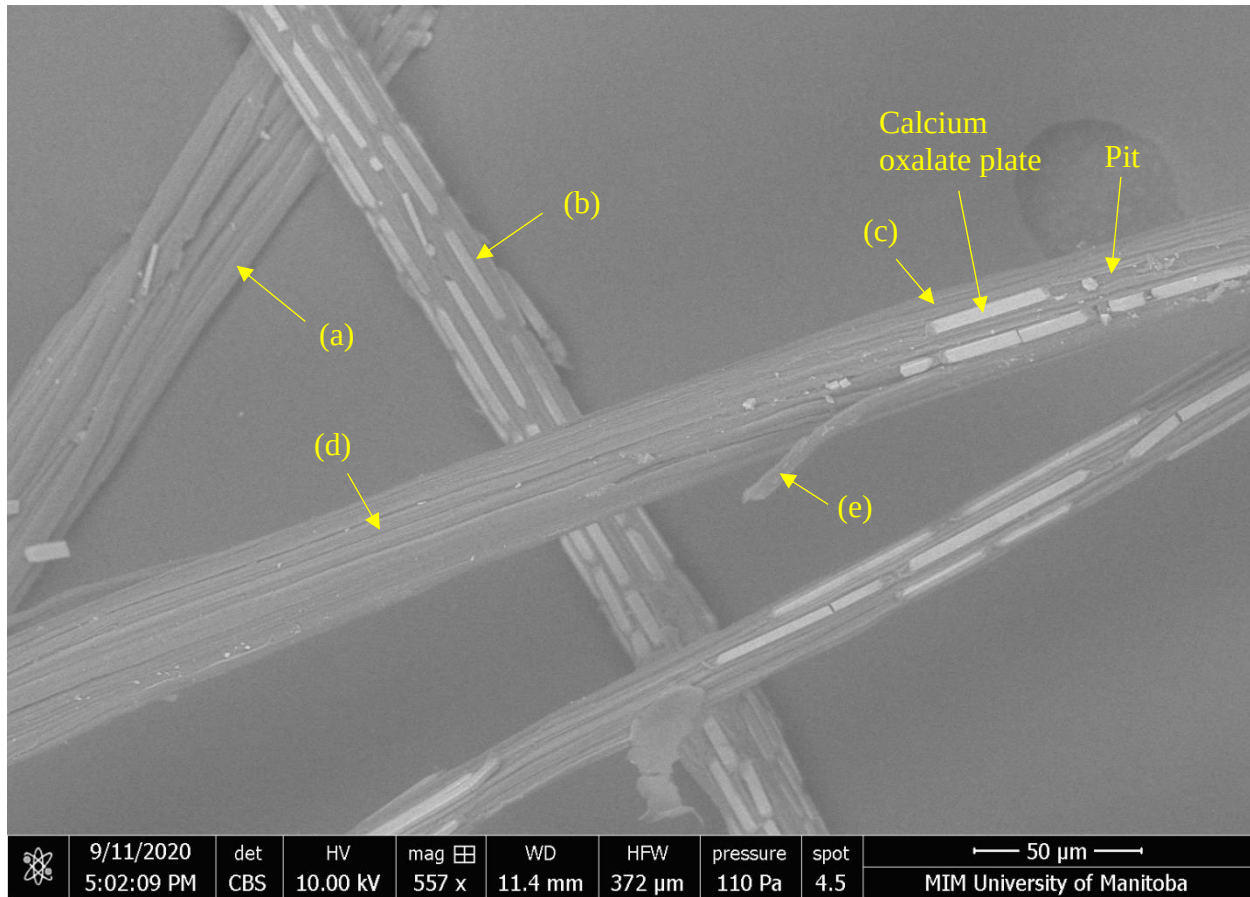


Figure 6.2.1.11. A backscattered electron image showing the fibers with different morphology. (a). The division of the smaller diameter structural fibers became clear. They appeared to be a less ordered bundle than (d). (b). The less structurally dissociated fiber, which had many calcium oxalate plates on the surface. (c). The section of the fiber where the calcium oxalate plate and pit could be found. (d). The section of the fiber where the surface morphology of pit was not obvious or was different from (c), while large calcium oxalate plates could not be found on this section of fiber. (e). The section of the structural fiber branched from the larger diameter fiber or fiber bundle. But note that the relation between the surface morphology and the mechanical properties was outside the scope of this research.

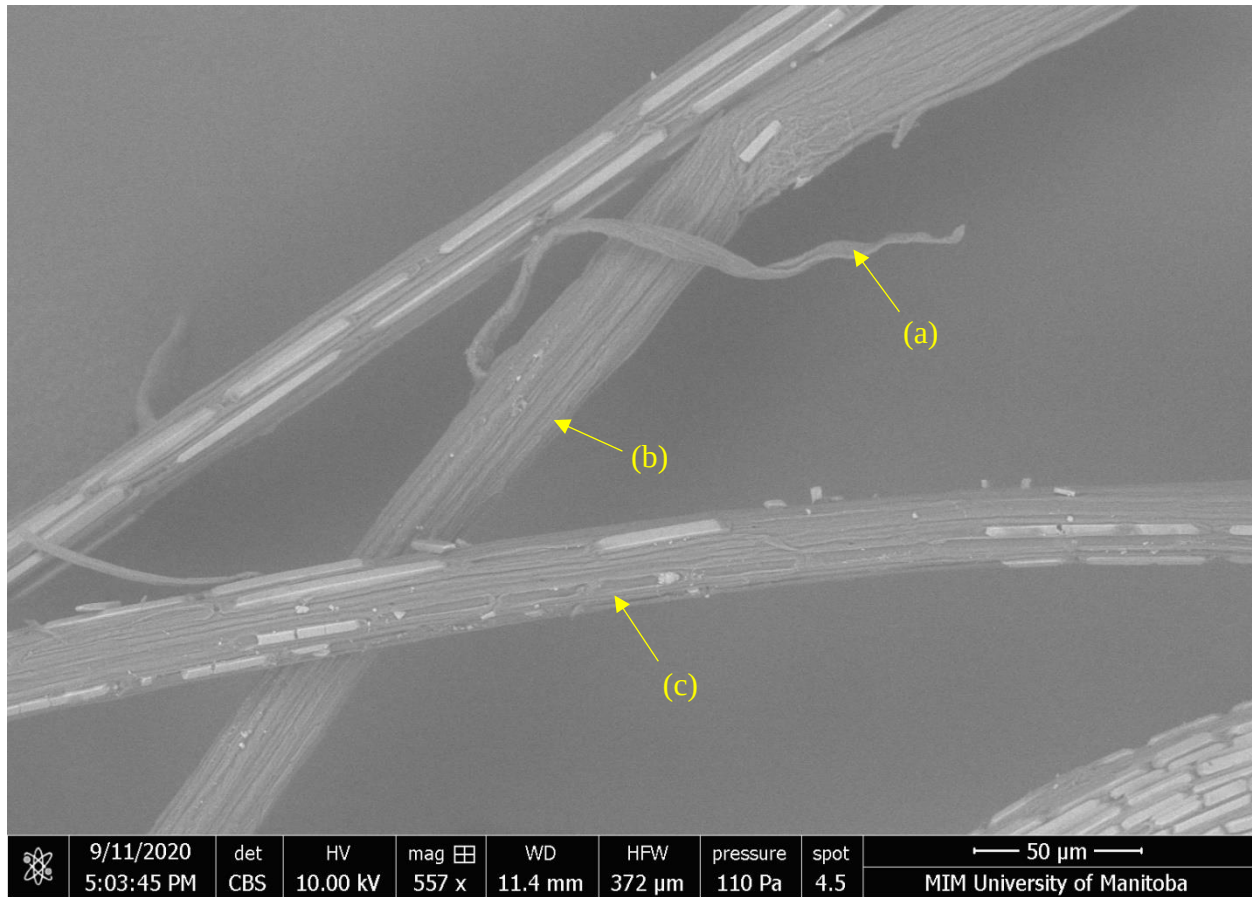


Figure 6.2.1.12. A backscattered electron image showing the fibers with different morphology. A section of a structural fiber, (a) branched from the larger diameter fiber or fiber bundle, (b). The fiber indicated by (b) was more chemically extracted than (c) because the fiber indicated by (c) had more calcium oxalate plates and the surface morphology of pit was more obvious than or different from (b). The relation between the extent of chemical extraction and the surface morphology was only a speculation and it was uncertain whether the post-chemical extraction handling process and the number of types of fiber would affect the surface morphology.

6.2.2 Calcium oxalate plates and pits

In order to develop the application of cattail leaf fibers, the morphological characteristics of the extracted fiber product have to be examined thoroughly. The calcium oxalate plate on the fiber surface is certainly a factor that requires investigation. An EDS test was carried out on the surface which had different morphology of a chemically extracted fiber (same extraction condition as the sample in section 6.2.1) to examine the calcium oxalate plate, the remainder of the calcium oxalate plate, the pit, and the non-plate area of their elemental composition. This could provide more information on how the calcium oxalate plates were attached to the surface. Because in the future, it is very likely that these calcium oxalate plates have to be removed during the extraction process or to apply separately a process to remove the calcium oxalate plates for rendering the fiber more suitable for an application (although this should not be the only factor to consider when determining a suitable application). The EDS test could check the calcium atomic % of the pit after a calcium oxalate plate was removed. But this removal was not planned here because the systematic removal of the calcium oxalate plates and the confirmation of their clearance were not within the research scope of this thesis, but this research can provide some insight on the appearance of the fiber surface after the calcium oxalate plates were removed.

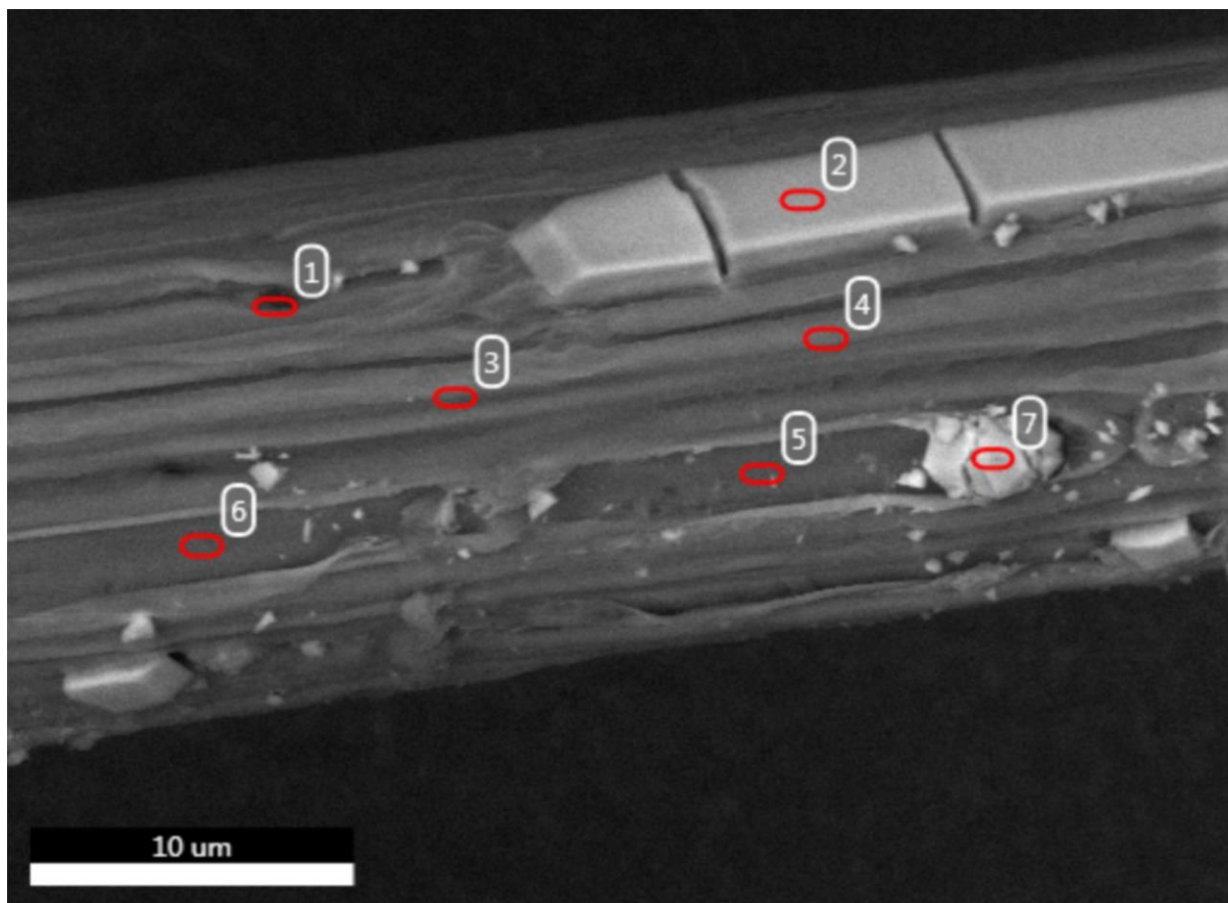


Figure 6.2.2.1. A backscattered electron image with a larger magnification zoomed in on Figure 6.2.1.12. The sample was a chemically extracted fiber. Label 1 – 7 indicated the detection focus areas (with red sides) of the energy-dispersive X-ray spectroscopy. Label 2 focused on the calcium oxalate plate; label 7 focused on the remainder of the calcium oxalate plate; label 1, 5, and 6 focused on the pits; label 3 and 4 focused on the non-plate areas.

Table 6.2.2.1. EDS results on a chemically extracted fiber

Label	Surface morphology	Ca atomic %	C atomic %	O atomic %
1	The pit	0.9	81.6	17.0
2	The calcium oxalate plate	8.6	61.7	29.6
3	The non-plate place	0.8	81.4	17.3
4	The non-plate place	0.9	81.7	17.1
5	The pit	1.1	82.1	16.7
6	The pit	1.0	81.9	17.0
7	The remainder of the calcium oxalate plate	9.1	64.6	26.2

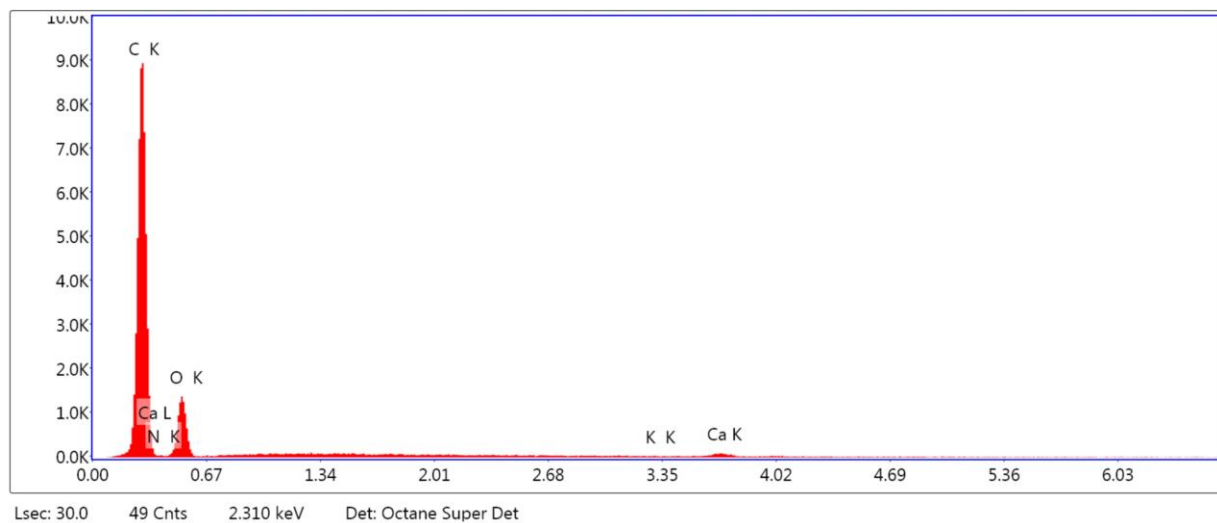


Figure 6.2.2.2. EDS results for label 1 (the pit) in Figure 6.2.2.1.

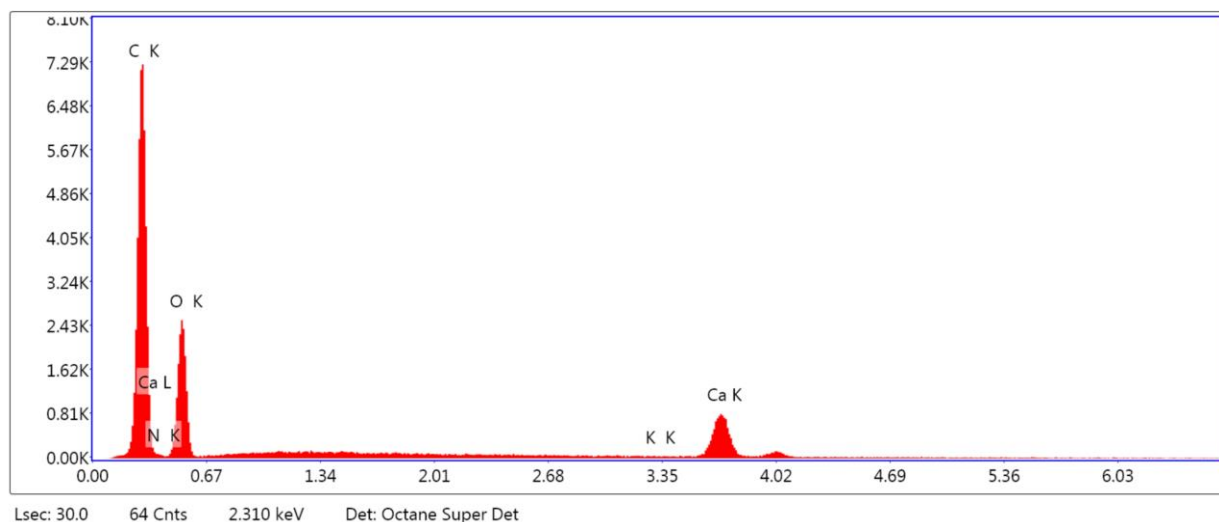


Figure 6.2.2.3. EDS results for label 2 (the calcium oxalate plate) in Figure 6.2.2.1.

The pits had Ca atomic % close to the non-plate places than the calcium oxalate plate and the remainder of the calcium oxalate plate. Debris could be found in the pits.

6.2.3 Color

Color can be a factor in fiber selection for a particular purpose. Experiments were carried out to investigate whether different parts of cattail leaves would produce different colors at the end of the chemical extraction process.



Figure 6.2.3.1. A photo showing different parts of Typha leaves before chemical extraction. Left: lamina; middle: sheath; right: sheath with the ventral and dorsal surface removed.

In Figure 6.2.3.1, before the leaf samples were immersed in the alkaline solution in an elevated temperature, they could be observed visually with different colors. Three types of samples were tested. The first two, left and middle, which were the lamina and sheath could be prepared in a relatively easier way because they were just upper and lower part of the leaves, while the sheath part with the ventral and dorsal surface removed would require more effort in the preparation. The easiness of raw material processing and resulting fiber colors can become part of the consideration factors for the industrial development in the future.

The lamina, sheath, and sheath with the ventral and dorsal surface removed (Figure 6.2.3.1) were extracted chemically. The sheath samples were examined using ESEM and EDS, and the results were presented in section 6.1. The extraction conditions of the lamina and sheath with the ventral and dorsal surface removed were similar to that of the sheath samples, but note that the sample to alkaline solution mass ratios were not the same for these three samples.



Figure 6.2.3.2. A photo showing fibers extracted from different parts of Typha leaves by chemical extraction. Left: fibers from lamina; middle: fibers from sheath; right: fibers from sheath with the ventral and dorsal surface removed. Different colors could be observed.

Figure 6.2.3.2 showed the fiber products after the chemical extraction. Each type of the extracted fibers was found to have different color. By visual observation, fibers from the lamina had darker brown color; fibers from the sheath had lighter brown color; fibers from the sheath with ventral and dorsal surface removed had yellower color. But whether the sample to alkaline solution mass ratio would also have an effect to the color difference was not investigated. Literature has reported that fibers from different parts of a *Typha* leaf had different properties and this would be

discussed in chapter 7. Understanding more about the fibers obtained from different leaf parts may allow a more selective extraction process for producing fibers with more satisfying properties.

CHAPTER 7. Comparison between different types of extracted fibers and the discussion of *Typha* leaf fiber extraction

7.1 Comparison between different types of extracted fibers

7.1.1 Mean and variance of diameters

Table 7.1.1.1. Diameter measurement results and statistical data

Extraction method	Mean diameter (μm)	Standard deviation (μm)	Variance (μm^2)
Manual extraction	49.4	8.8	77.3
Manual extraction. After that, chemical treatment at room temperature	30.6	2.1	4.3
Manual extraction. After that, chemical treatment at 70 – 80°C	29.5	4.8	22.9

The variance of diameters of the manually extracted fibers: V_1

The variance of diameters of the chemically treated (at room temperature) manually extracted fibers: V_2

The variance of diameters of the chemically treated (70 – 80°C) manually extracted fibers at: V_3

Table 7.1.1.2. F-test results

P-value	Result (the variance)
$1.268 \times 10^{-7} < 0.05$	$V_1 > V_2$
$0.01552 < 0.05$	$V_1 > V_3$
$0.0009395 < 0.05$	$V_2 < V_3$

The mean of diameters of the manually extracted fibers: M_1

The mean of diameters of the chemically treated (at room temperature) manually extracted fibers:
 M_2

The mean of diameters of the chemically treated (at 70 – 80°C) manually extracted fibers: M_3

Table 7.1.1.3. Welch two sample t-test results

P-value	Result (the mean)
$2.028 \times 10^{-8} < 0.05$	$M_1 > M_2$
$4.349 \times 10^{-9} < 0.05$	$M_1 > M_3$
$0.4362 > 0.05$	The difference between M_2 and M_3 was not statistically significant

The diameters of nodes were not included in the data (Table 7.1.1.1 - 7.1.1.3) and the description of the statistical analysis could be referred to Appendix IV.

The manual approach extracted lacunae fibers as the target fibers. These fibers, except the surface morphology of nodes and calcium oxalate plates, were in a relatively more regular morphology. After they were chemically treated with the potassium hydroxide solution at room temperature or 70 – 80°C, their mean diameters and variances of diameters were reduced. Besides, chemical treatment at 70 – 80°C (as well as a lower sample to alkaline solution mass ratio) did not change the mean diameter of the chemically treated (at room temperature) manually extracted lacunae fibers in a statistically significant manner. Furthermore, the variances were different for the chemically treated lacunae fibers at different temperatures. It was uncertain about this point. Some factors were listed here, but it was not clear whether they were critical to this difference: these two experiments had different sample sizes in the statistical analysis, no restriction was made to ensure that a longer lacunae fiber was not cut into more than one shorter lacunae fiber and no restriction for not to consider these shorter lacunae fibers as different fiber samples, different types

of microscope (optical microscope and ESEM) were used and the measurement setup was different, *Typha* species were not strictly monitored, and different sample to alkaline solution mass ratio.

7.1.2 Surface morphology

The products obtained from the extraction processes were mainly “fiber-like” with the length considerably longer than the width, but this was not enough for comparing their surface morphology. Three factors were considered in the comparison. Firstly, their structural morphology could be considered. The fibers in the manually extracted fiber product were mainly of a similar type (lacunae fibers) and structure. The chemically treated manually extracted fibers also showed this characteristic. For the chemically extracted fibers produced by using the protocol in this thesis, diameters with observably large differences (Figure 6.2.1.1 and 6.2.1.5) could be found. The formation of larger diameter fibers in the product could possibly be due to bundling structures with unreacted connective materials or different types of fibers, which would be discussed in the later section.

Secondly, the extent of calcium oxalate plates present on the surface was different. For the manually extracted lacunae fibers and their chemically treated form, the calcium oxalate plates (Figure 4.2.1.2, 5.2.1.2, 5.2.1.4, 5.2.1.5, 5.4.1.2, and 5.4.1.3) were more regularly present than the chemically extracted fibers (Figure 6.2.1.11 and 6.2.1.12). Some chemically extracted fibers (or sections of them) had shown a surface with the calcium oxalate plates mostly removed, while for some other fibers (or sections of them), calcium oxalate plates were present on their surfaces with different amount.

Thirdly, the node. The manually extracted lacunae fibers (Figure 4.2.1.1 and 4.2.1.3) and their chemically treated form (Figure 5.2.1.1, 5.2.1.2, 5.2.1.3, and 5.4.1.1) showed the node morphology. But it was inconclusive for the chemically extracted fibers.

7.2 Discussion

7.2.1 The diameter problem

A critical problem for the fibers obtained from the chemical extraction was the high variation in diameter. There were difficulties in determining the diameters of the fiber samples because they could be in a bundled form. When researchers observed a bundling structure, which branched at a position, there would be a question of how the diameter could be measured.

If the fibers are manually separated and then their diameters are measured, the measured data are actually showing the diameter of the post-chemical extraction processed fibers, because one more step has been taken and should not be ignored. This step should be investigated and a protocol should be made.

If the diameter of fiber bundles and also fibers which branch from the bundles are measured, a large variation of the diameters will be expected. But the problem still exists because it is difficult to determine the part of the bundle to be included in the measurement.

A solution to this problem is to obtain more regular fibers with fewer bundles. Optimizing the chemical extraction protocol may help improve the fiber quality. But understanding the structure is also important. The alkaline solution used in the chemical extraction basically reacts with the entire leaf, which involves the inner and outer part of the leaf. A speculation for the chemical extraction experiment in this research was that the reaction was not regular throughout the leaves and caused the final product to have fibers of different structures. Changing the chemical

extraction protocol with stronger conditions, for example, a higher chemical concentration, higher temperature, longer extraction time, etc., may eventually remove all the outer structural materials. This research did not conclude whether stronger conditions can successfully produce more regular fibers, but other factors, for example, economic value, may change. If the extraction method is changed from chemical to mechanical, it is possible that the fibers can directly be obtained, but further processing is still necessary.

The mean and variance of diameters ($29.5 \pm 4.8 \mu\text{m}$ with the CV%, 16.2%) of the manually extracted lacunae fibers with the chemical treatment at $70 - 80^\circ\text{C}$ were lower than that of the reported data ($46.27 \pm 16.83 \mu\text{m}$ with the CV%, 36.38%) which was one of the data in the thesis of Chakma et al. (2018) and they obtained this result from their chemical extraction protocol and random sampling of *Typha* fiber. A possible explanation of this difference was that fibers of a similar type (lacunae fibers) were separated from the surrounding structures and selectively obtained from the sheath part of *Typha* leaves in the manual extraction (details could be referred to section 4.1). An important characteristic of the lacunae fibers in the leaf sheath was that they were not tightly embedded in the structure and could be taken out from the leaf.

7.2.2 The fibers obtained from the chemical extraction

Based on the research of this thesis, some arguments can be considered when discussing whether the fibers obtained from the chemical extraction are the lacunae fibers. However, no solid conclusion can be obtained as more investigations and proofs were required.

It can be deduced that chemically extracted fibers and manually extracted lacunae fibers should have difference because the former has reacted with the alkaline solution but the latter has not. The experiments have shown that if the manually extracted lacunae fibers are put in the

alkaline solution, their mean diameter will be reduced. This means that the fibers have reaction with the alkaline solution and parts of their components are removed. It is interesting to know whether the fibers obtained from the chemical extraction are the chemically treated form of the lacunae fibers.

Although in the previous sections, the diameter and surface morphological difference between the chemically extracted fibers and manually extracted lacunae fibers with chemical treatment were shown, there were possibilities that the chemically extracted fibers with different diameters, bundles with structural fibers separated, and calcium oxalate plates removed in different extent were the results of an uneven chemical reaction. Those reacted less might still include some structural materials like aerenchyma and have more calcium oxalate plates on their surfaces. An important observation was that lacunae fibers had calcium oxalate plates on their surfaces, while many chemically extracted fibers had these plates on their surfaces in different extent. A speculation was that lacunae fiber was one of the main types of fibers of chemical extraction, but whether it was the only type of extracted fiber was not clear. It was possible that the fiber products included more than one type of fiber and lacunae fiber was only one of them. So, it is necessary to understand the structure of the leaves.

From the botanical perspective (the fiber in the literature should be referring to a more botanically defined fiber, instead of the definition of fiber in this thesis), the lamina margin of *T. angustifolia* was reported to have a thick zone of fibers and vascular bundles could also be found at the proximal edge of it, while the subepidermal vascular bundles along the abaxial and adaxial margins were interspersed with fiber bundles in the chlorophyllous mesophyll (McManus et al. 2002). Besides, it was reported that the dorsal and ventral surface tissues and the partitions of *T. domingensis* leaf blade contained lignified vascular bundles (Witztum and Wayne 2016a).

Considering the alkaline extraction, the structural materials were expected to be removed by the chemical, but was the lacunae fiber the only kind of fiber left in the chemical extraction process? Or could any of the above-mentioned leaf materials in the literature remain unremoved after the process? Based on the ESEM images (section 6.2.1) obtained in this research, many chemically extracted fiber products had the calcium oxalate plates as their surface morphology with different extent. Many fibers with different amount of calcium oxalate plates and some even showing the smaller diameter structural fibers were potentially the lacunae fibers extracted or reacted at different levels. Therefore, it is necessary to know whether other leaf materials apart from the lacunae fiber also consist of calcium oxalate plates as their surface morphology or only lacunae fiber does. However, these were not clear in this research and therefore, the only deduction which could be made in terms of the fiber type was that the fiber product obtained from the chemical extraction using the protocol in this research should include lacunae fiber, but not clear about whether it was the only type of extracted fiber or not.

7.2.3 The target fiber structure

Apart from the fiber type, researchers should also examine the target fiber structure in an extraction process. Chemical extraction can produce “fiber-like” materials. However, the CV% for the diameter can be relatively large. The potentially uneven chemical reaction for the entire leaves may be one of the causes of this result. To solve this problem, additional processing methods to improve the fiber diameter should be developed, but alternatively, lacunae fibers can be extracted manually at the beginning of the extraction process. The manually extracted lacunae fibers can have more regular diameters than the chemically extracted fibers which are extracted using certain

protocols. After the manual extraction, the lacunae fibers can be chemically treated to give a smaller diameter and SD.

In the chemical extraction process, some structures observed in this research were 1. fibers with surrounding structural materials (e.g. aerenchyma), 2. fibers with different amount of calcium oxalate plates on the surfaces, 3. the surface morphology of pits of the fibers were not obvious or were changed, most of the calcium oxalate plates were removed, and the surface morphology of smaller diameter structural fibers became obvious, 4. the bundle structurally dissociated; the smaller diameter structural fibers in the bundle separated from each other. A speculation was that if fibers react more with the chemicals, they may tend to be more structurally dissociated. However, the post-chemical extraction process and the number of types of fibers, which were not clear in the fiber product might also be the causes of these morphological differences and they should be further investigated. Nevertheless, a suitable structure should be targeted in an extraction process. If the product of extraction consists of fibers with different structural morphology, it can be expected that the quality will be lower.

7.2.4 The surface morphology problems

Calcium oxalate plates may limit the application of *Typha* leaf fibers. Researchers should expect that additional processes are necessary to completely remove these plates and ensure that these plates are removed (not only the plates but also the debris). This research has shown that pits were left on the surface of the fibers after the calcium oxalate plates were removed. Table 6.2.2.1 has shown that the EDS results of the plate, the remainder of the plate, the pits and the non-plate places. The Ca atomic % might indicate more about the presence of calcium oxalate plate. The pits had Ca atomic % closer to the non-plate places than the calcium oxalate plate and the remainder

of the plate. In Figure 6.2.1.11, (d) showed the surface morphology of the pits were changed or less obvious than (c). In Figure 6.2.1.3, for the thinner fiber with red arrowheads pointing to the pits, these pits were obvious and might have been previously occupied by calcium oxalate plates. A speculation was that if a fiber (or lacunae fiber) which has calcium oxalate plates as its surface morphology is more reacted, the morphology of the pits may change and the calcium oxalate plates may be detached from the pits. A more reacted lacunae fiber might be less like Figure 6.2.1.3 and become more similar to Figure 6.2.1.11 (d). However, this was only a speculation because it was not clear whether the post-extraction process and the number of types of extracted fiber might have any effect on the morphological results.

Apart from that, the nodes in the manually extracted lacunae fibers may also limit the application. For the chemically treated manually extracted lacunae fibers, nodes and calcium oxalate plates could still be found on the surface. Chemical treatment had an effect to reduce the mean and variance of diameters of the manually extracted lacunae fibers, but its effect on the nodes and calcium oxalate plates could not be concluded in this research.

7.2.5 The significance of the post-extraction process

Studying the post-extraction processes can be helpful because important steps may have been involved. In the chemical extraction protocol of this thesis, the extracted fibers were separated using a glass rod, which was a post-extraction process. It could be observed that there were smaller diameter structural fibers separated at the loop (Figure 6.2.1.7 and 6.2.1.8) and bend (Figure 6.2.1.9) of the chemically extracted fibers, but it was difficult to tell whether 1. they reacted more with the chemical and the reaction dissociated the structure, 2. the formation of loop and bend physically dissociated the structure, or 3. the post-extraction process dissociated the structure. Although the

causes were not clear, these might be insightful to the investigation and development of post-extraction processes, which might improve the fiber quality. For the manual extraction, the chemical treatment, which reduced the mean and variance of diameters (section 7.1.1) could also be considered as a post-extraction process (depending on the definition of it). Formulating post-extraction processes may provide more options in controlling the fiber diameter and producing fibers with higher quality, but it is crucial to also investigate how the mechanical properties will be affected by these post-extraction processes.

7.2.6 Fiber extraction of different parts of a leaf

When extracting fibers, choosing different leaf part (sheath or lamina) will make a difference. Witztum and Wayne (2016b) investigated *Typha angustifolia* L. and found that the lacunae fiber cables in the leaf blade and leaf sheath were continuous, but not all lacunae fiber cables in the leaf sheath would extend into the leaf blade. There were fewer lacunae fiber cables in the leaf blade than in the leaf sheath. Besides, Rowlatt and Morshead (1992) reported that in *Typha latifolia* L., the continuous internal structure involved only gradual changes between the sheath and lamina. The stellate cell wisps were abundant in the sheath, the amount became fewer gradually in the lamina, and they were absent in the distal lamina. For the research in this thesis, section 6.2.3 showed that the sheath and lamina would produce fiber products with different colors. Therefore, based on these findings, it could be understood that fewer lacunae fibers might be obtained if the lamina part was extracted; while there might be more lacunae fibers obtained when extracting the sheath part, more non-diaphragm aerenchyma would be expected; different colors of fiber products could be obtained from the sheath and lamina for the chemical extraction.

For the manual extraction, it was discussed in section 4.3 that lacunae fibers could be extracted from the sheath part more easily than the lamina part as cutting the dorsal surface of the lamina was more difficult.

Witztum and Wayne (2016b) used phloroglucinol-HCl to examine the lignin for the lacunae fiber cables of *Typha angustifolia* L. They found that the lacunae fiber cables were lignified in the sheath, but not lignified in the leaf blade. The lignified lacunae fiber cables were measured to have a larger diameter than the non-lignified lacunae fiber cables. They also found differences in the mechanical properties between the lacunae fiber cables in the sheath and leaf blade.

Therefore, choosing a different leaf part for the extraction is very important for both manual and chemical extraction approach.

CHAPTER 8. Conclusions

Typha leaf fibers can be very useful resources in the fiber engineering field. They are potentially a low-cost material if they can be suitably applied in a certain industry. However, this has not been achieved yet. Part of the reasons might be that its very fundamental characteristics have not been determined in the research field and its application also could not be positioned well because many properties remain uncertain. Researchers in this field need to understand the target fibers of their own extraction methods. Chemical extraction is one of the current options for extracting the *Typha* leaf fibers. The main idea is to remove the structural materials and dissociate the leaf structures in order to separate the fibers. But depending on the application, the fiber diameters might not satisfy the purpose and the CV% could also be high.

Different structures were found in the chemical extraction experiment: fibers with the surrounding leaf materials; isolated fibers with calcium oxalate plates on their surfaces, while the amount of calcium oxalate plates could be different; calcium oxalate plates were detached and pits could be observed as the surface morphology; the surface morphology of pits became less obvious or changed and the calcium oxalate plates were detached, leaving fibers with surface morphology showing the structural fibers with smaller diameters bundled together; and bundles of structural fibers which were separated from each other. Other morphology could also be observed in this research. A possible explanation for these structures was that different reaction extent for the leaves might produce fibers with different structural morphology. But apart from different reaction extent, the post-chemical extraction process and the number of types of fiber might also be the causes of these observed morphological differences. It could not be certain how many types of fiber were present in these structures. It could be observed that a number of fibers had calcium

oxalate plates on their surfaces. A deduction was made based on this observation that lacunae fiber was one type of fiber in the chemical extraction process. This research could not find out whether lacunae fiber was the only fiber type in the chemical extraction process as there were different leaf materials in a leaf and more investigation is required to better understand this aspect.

If lacunae fibers are important in *Typha* leaf extraction, then directly and physically extracting them from the leaves can be an alternative, but chemical treatment will still be necessary. Lacunae fibers could be obtained by removing the surrounding structural materials manually. A method was developed in this thesis to manually separate the fibers from the leaves. A consistent type of fiber could be obtained using this method, except the node and calcium oxalate plate problems. The manual extraction process was an attempt to avoid other leaf materials and aimed to only obtain the lacunae fibers. Lacunae fibers fundamentally had a more regular diameter and CV%. Further treatment with certain conditions on these fibers could form products also with regular diameter and CV%. In this research, alkaline chemical treatment was done on the manually extracted lacunae fibers, the mean diameter $\pm$ SD and CV% changed from $49.4 \pm 8.8 \mu\text{m}$, 17.8% to $30.6 \pm 2.1 \mu\text{m}$, 6.9% at room temperature and $29.5 \pm 4.8 \mu\text{m}$, 16.2% at 70 – 80°C (these data did not include the measurement of nodes). The mean and variance of diameters were lowered after the chemical treatment at both room temperature and 70 – 80°C. However, the main problems were the surface morphology of nodes and calcium oxalate plates. The node might be the remainder of the diaphragm. Further investigation should be carried out on the surface morphology of the fibers.

The main difference between the manual and chemical extraction method was that the manual approach could get relatively more regular fibers with nodes and calcium oxalate plates. The use of potassium hydroxide solution could reduce the mean and variance of diameters of the manually extracted lacunae fibers. The chemical extraction might produce fibers with different

extent of extraction and they could potentially be less regular. Further treatment was required for both extraction methods to remove the nodes and calcium oxalate plates if they were not removed in the extraction process. The post-chemical extraction process, which might also affect the fiber quality should be studied. People who investigate *Typha* leaf fiber extraction should pay attention to the leaf parts where they extract the fibers. This research also found that lamina, sheath, and sheath with the ventral and dorsal surface removed would produce fiber products with different colors in chemical extraction. Understanding the difference of the fibers at different leaf parts may allow a more selective extraction to obtain fibers with more satisfactory properties.

A potential research area involves the design of machines for a mechanical extraction because firstly, manual extraction has a low extraction efficiency. For example, in step 3 of the manual extraction method (Figure 4.1.8) about the ventral surface removal, the extraction process could be simplified if both dorsal and ventral surface were removed in step 1. However, unlike the dorsal surface which after it was flattened and the thinner sides were removed could be cut easily to separate, the process for removing the ventral surface manually in a large slice at a time was delicate; secondly, the yield percentage, $(\text{mass of the extracted lacunae fibers} / \text{mass of the leaf}) \times 100\%$ tends to be lower because some of the extractable lacunae fibers can be wasted during the extraction process. A mechanical extraction using machines with an automated process may be able to increase the efficiency and yield percentage. Moreover, it is important to investigate the influence of nodes and calcium oxalate plates. For example, to examine whether they will cause adverse effect in some fiber applications.

References

- Anderson, S. L., McGranahan, D. A., Hovick, T. J., & Hewitt, A. R. (2019). Passerine and secretive marsh bird responses to cattail management in temperate wetlands. *Wetlands ecology and management*, 27(2-3), 283-293.
- Bansal, S., Lishawa, S. C., Newman, S., Tangen, B. A., Wilcox, D., Albert, D., Anteau, M. J., Chimney, M. J., Cressey, R. L., DeKeyser, E., Elgersma, K. J., Finkelstein, S. A., Freeland, J., Grosshans, R., Klug, P. E., Larkin, D. J., Lawrence, B. A., Linz, G., Marburger, J., Noe, G., Otto, C., Reo, N., Richards, J., Richardson, C., Rodgers, L., Schrank, A. J., Svedarsky, D., Travis, S., Tuchman, N., & Windham-Myers, L. (2019). *Typha* (Cattail) invasion in North American wetlands: Biology, regional problems, impacts, ecosystem services, and management. *Wetlands*, 39(4), 645-684.
- Carranza-Álvarez, C., Alonso-Castro, A. J., Alfaro-De La Torre, M. C., & García-De La Cruz, R. F. (2008). Accumulation and distribution of heavy metals in *Scirpus americanus* and *Typha latifolia* from an artificial lagoon in San Luis Potosí, México. *Water, air, and soil pollution*, 188(1-4), 297-309.
- Chakma, K. 2018. Extraction efficiency, quality and characterization of *Typha latifolia* L. Fibres for textile applications. M.Sc. thesis. Winnipeg, Manitoba: Department of Biosystems Engineering, University of Manitoba.
- Chen, Y. L., Hong, X. Q., He, H., Luo, H. W., Qian, T. T., Li, R. Z., Jiang, H., & Yu, H. Q. (2014). Biosorption of Cr (VI) by *Typha angustifolia*: Mechanism and responses to heavy metal stress. *Bioresource technology*, 160, 89-92.

- Constable, J. V. H., & Longstreth, D. J. (1994). Aerenchyma carbon dioxide can be assimilated in *Typha latifolia* L. leaves. *Plant Physiology*, 106(3), 1065-1072.
- Dong, T., Xu, G., & Wang, F. (2015). Oil spill cleanup by structured natural sorbents made from cattail fibers. *Industrial Crops and Products*, 76, 25-33.
- Grace, J. B., & Harrison, J. S. (1986). The biology of Canadian weeds. 73. *Typha latifolia* L., *Typha angustifolia* L. and *Typha xglauca* Godr. *Canadian Journal of Plant Science*, 66(2), 361-379.
- Leto, C., Tuttolomondo, T., La Bella, S., Leone, R., & Licata, M. (2013). Effects of plant species in a horizontal subsurface flow constructed wetland–phytoremediation of treated urban wastewater with *Cyperus alternifolius* L. and *Typha latifolia* L. in the West of Sicily (Italy). *Ecological engineering*, 61, 282-291.
- Linz, G. M., & Homan, H. J. (2011). Use of glyphosate for managing invasive cattail (*Typha* spp.) to disperse blackbird (Icteridae) roosts. *Crop Protection*, 30(2), 98-104.
- Luamkanchanaphan, T., Chotikapraphan, S., & Jarusombati, S. (2012). A study of physical, mechanical and thermal properties for thermal insulation from narrow-leaved cattail fibers. *APCBEE Procedia*, 1, 46-52.
- Martin, A. C., Zim, H. S., & Nelson, A. L. (1961). American wildlife & plants: A guide to wildlife food habits: the use of trees, shrubs, weeds, and herbs by birds and mammals of the United States. Courier Corporation.
- McDougall, G. J., Morrison, I. M., Stewart, D., Weyers, J. D. B., & Hillman, J. R. (1993). Plant fibres: Botany, chemistry and processing for industrial use. *Journal of the Science of Food and Agriculture*, 62(1), 1-20.

- McManus, H. A., Seago Jr, J. L., & Marsh, L. C. (2002). Epifluorescent and histochemical aspects of shoot anatomy of *Typha latifolia* L., *Typha angustifolia* L. and *Typha glauca* Godr. *Annals of Botany*, 90(4), 489-493.
- Molnár, Á., Hegedüs, R., Kriska, G., & Horváth, G. (2011). Effect of cattail (*Typha* spp.) mowing on water beetle assemblages: changes of environmental factors and the aerial colonization of aquatic habitats. *Journal of Insect Conservation*, 15(3), 389-399.
- Mwaikambo, L. Y., & Ansell, M. P. (2002). Chemical modification of hemp, sisal, jute, and kapok fibers by alkalization. *Journal of applied polymer science*, 84(12), 2222-2234.
- Nilratnisakorn, S., Thiravetyan, P., & Nakbanpote, W. (2007). Synthetic reactive dye wastewater treatment by narrow-leaved cattails (*Typha angustifolia* Linn.): Effects of dye, salinity and metals. *Science of the total environment*, 384(1-3), 67-76.
- Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <https://imagej.nih.gov/ij/>, 1997-2018.
- Rowlatt, U., & Morshead, H. (1992). Architecture of the leaf of the greater reed mace, *Typha latifolia* L. *Botanical journal of the Linnean Society*, 110(2), 161-170.
- RStudio Team (2016). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL <http://www.rstudio.com/>.
- Sana, R., Mounir, J., & Slah, M. (2014). Study of structure and properties of Tunisian *Typha* leaf fibers. *International Journal of Engineering Research & Technology (IJERT)*, 3.
- Smith, S. G. (1967). Experimental and natural hybrids in north American *Typha* (Typhaceae). *The American Midland Naturalist*, 78(2), 257-287.
- Wilson, P. C., Whitwell, T., & Klaine, S. J. (2000). Metalaxyl and simazine toxicity to and uptake by *Typha latifolia*. *Archives of environmental contamination and toxicology*, 39(3), 282-288.

- Witztum, A., & Wayne, R. (2014). Fibre cables in the lacunae of *Typha* leaves contribute to a tensegrity structure. *Annals of botany*, 113(5), 789-797.
- Witztum, A., & Wayne, R. (2015). Variation in fiber cables in the lacunae of leaves in hybrid swarms of *Typha* × *glauca*. *Aquatic botany*, 124, 39-44.
- Witztum, A., & Wayne, R. (2016a). Fiber cables in leaf blades of *Typha domingensis* and their absence in *Typha elephantina*: a diagnostic character for phylogenetic affinity. *Israel Journal of Plant Sciences*, 63(2), 116-123.
- Witztum, A., & Wayne, R. (2016b). Lignified and nonlignified fiber cables in the lacunae of *Typha angustifolia*. *Protoplasma*, 253(6), 1589-1592.
- Wuzella, G., Mahendran, A. R., Bätge, T., Jury, S., & Kandelbauer, A. (2011). Novel, binder-free fiber reinforced composites based on a renewable resource from the reed-like plant *Typha* sp. *Industrial Crops and Products*, 33(3), 683-689.

Appendix I – Data of the diameter measurement

Table A1.1. The diameter data for the manually extracted lacunae fibers (not including the nodes)

Sample	Trial 1	Trial 2	Trial 3
1	50.67	48.65	49.15
2	54.88	47.16	47.16
3	34.34	30.37	34.58
4	40.61	40.61	42.18
5	53.14	65.77	59.45
6	52.53	48.02	48.65
7	60.91	42.62	40.61
8	51.15	49.53	61.81
9	64.2	46.64	45.4
10	42.85	38.86	38.86
11	60.74	58.17	59.42
12	49.15	55.47	60.77
13	37.25	38.33	35.34
14	57.85	43.38	42.62
15	50.67	51.51	49.15
16	82.41	62.63	64.2
17	41.65	43.19	40.91
18	61.14	45.17	45.58

Table A1.2. The diameter data for the chemically treated (at room temperature) manually extracted lacunae fibers (not including the nodes)

Sample	Trial 1	Trial 2	Trial 3
1	26.95	31.62	28.07
2	31.94	32.57	31.62
3	32.64	34.76	32.57
4	29.41	29.41	31.94
5	35.34	32.64	34.34
6	26.34	28.35	26.34
7	37.14	30.84	32.57
8	30.03	25.31	27.48
9	32.64	33.44	31.36
10	24.66	31.56	31.36
11	28.07	30.9	31.94
12	30.77	30.03	28.92
13	24.99	24.58	34.4
14	35.8	33.86	28.92
15	31.94	30.37	30.03
16	31.94	28.92	31.94
17	27.48	28.92	33.62
18	29.14	31.62	30.3

Table A1.3. The diameter data for the chemically treated (at 70 – 80°C) manually extracted lacunae fibers (not including the nodes)

Sample	Trial 1	Trial 2	Trial 3
1	30.67	28.00	31.11
2	40.25	38.18	39.90
3	31.08	30.07	27.74
4	22.30	24.00	22.30
5	35.78	32.50	30.75
6	26.72	26.39	27.38
7	31.87	32.16	33.28
8	21.46	23.01	22.34
9	36.51	34.23	34.52
10	31.43	29.13	31.45
11	28.72	31.08	28.48
12	29.05	32.43	32.43
13	21.96	23.99	25.68
14	29.05	24.66	27.03

Appendix II – EDS results for labels in Figure 4.2.3.1

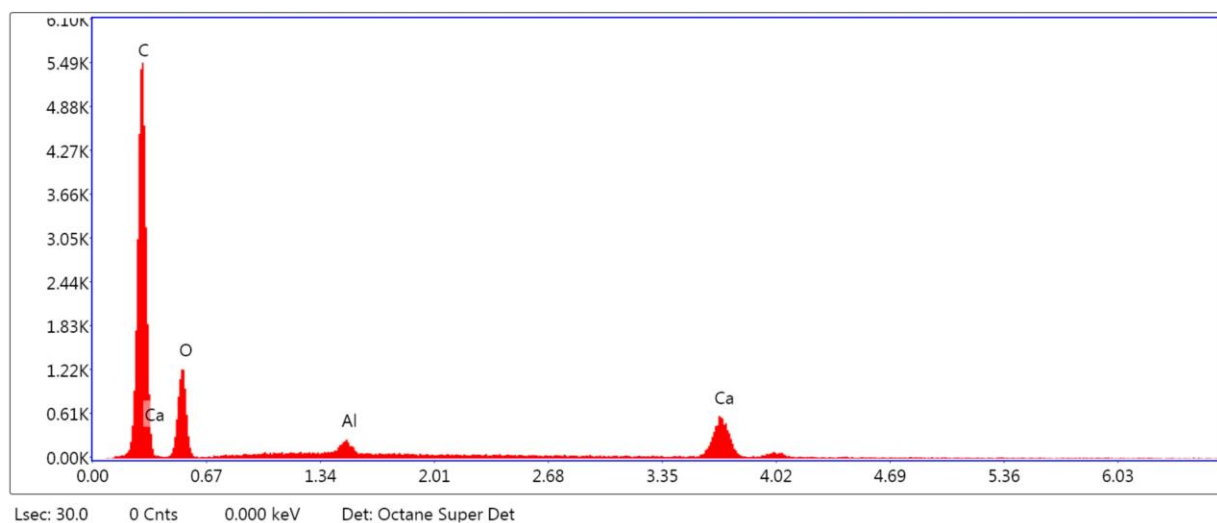


Figure A2.1. EDS results for label 1 (the plate) in Figure 4.2.3.1.

Table A2.1. EDS results for label 1 (the plate) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	54.6	69.0	4.7
O K	23.9	22.6	10.3
Al K	1.0	0.5	11.8
Ca K	20.6	7.8	5.5

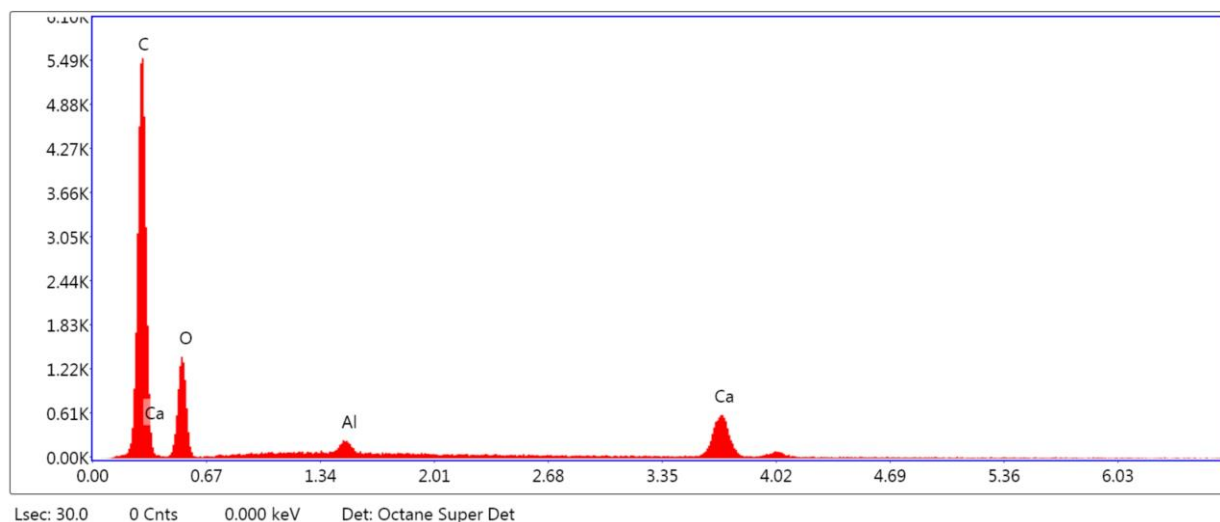


Figure A2.2. EDS results for label 2 (the plate) in Figure 4.2.3.1.

Table A2.2. EDS results for label 2 (the plate) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	52.8	67.2	4.8
O K	25.4	24.3	10.2
Al K	0.9	0.5	11.8
Ca K	20.9	8.0	5.5

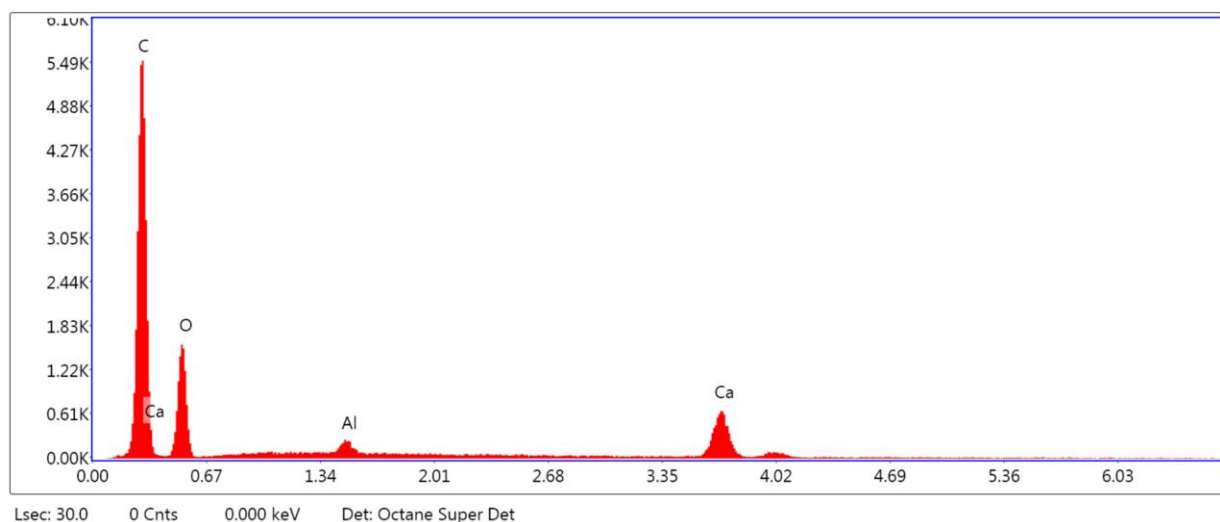


Figure A2.3. EDS results for label 3 (the plate) in Figure 4.2.3.1.

Table A2.3. EDS results for label 3 (the plate) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	51.4	65.6	4.8
O K	27.0	25.9	9.9
Al K	0.9	0.5	11.9
Ca K	20.8	8.0	5.4

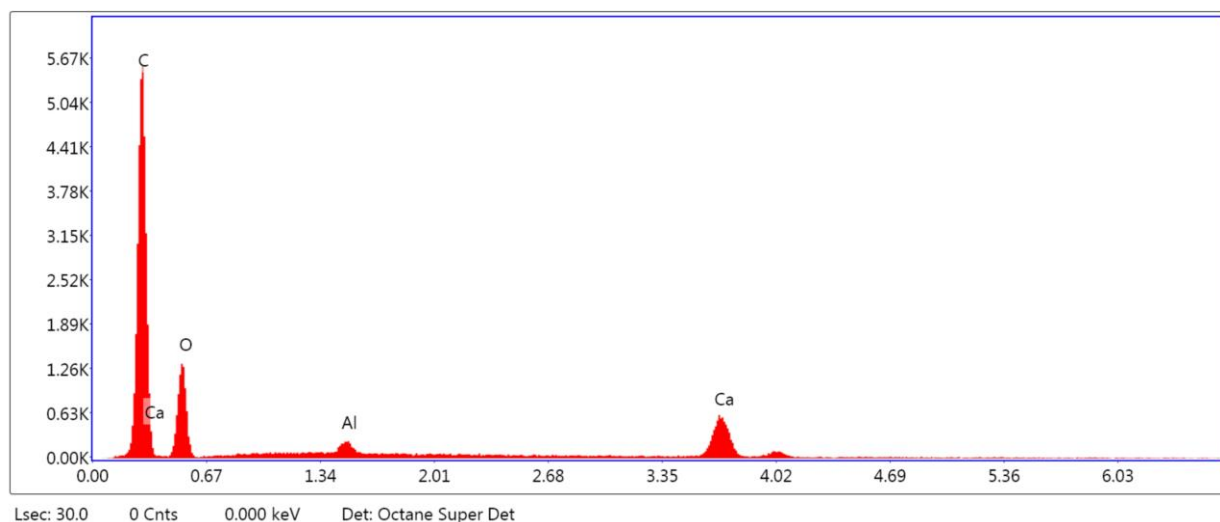


Figure A2.4. EDS results for label 4 (the plate) in Figure 4.2.3.1.

Table A2.4. EDS results for label 4 (the plate) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	53.1	67.6	4.8
O K	25.0	23.9	10.3
Al K	0.9	0.5	11.9
Ca K	21.0	8.0	5.5

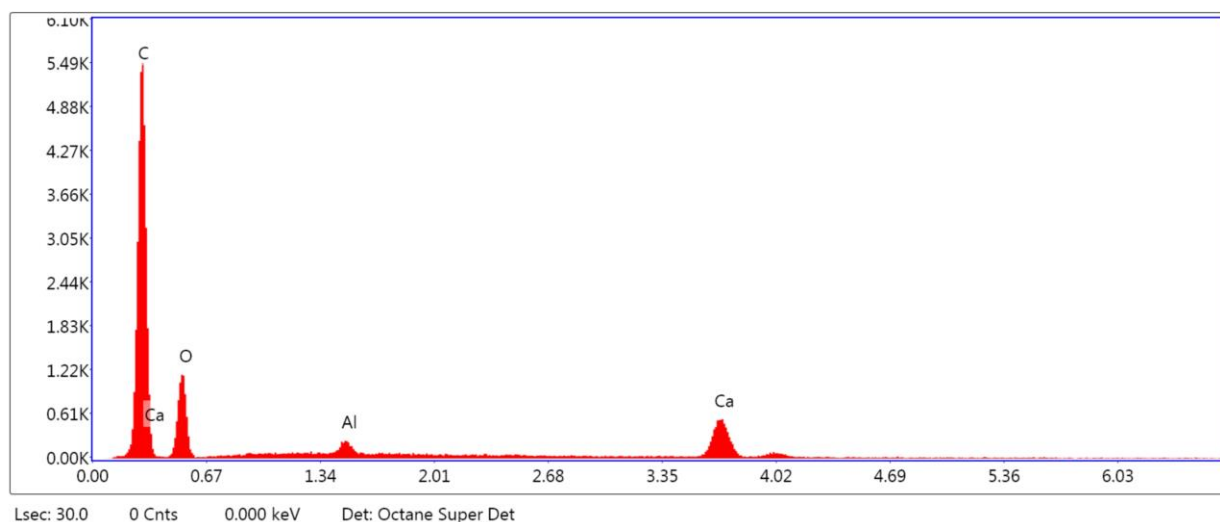


Figure A2.5. EDS results for label 5 (the plate) in Figure 4.2.3.1.

Table A2.5. EDS results for label 5 (the plate) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	55.5	69.9	4.7
O K	23.2	21.9	10.4
Al K	1.0	0.5	11.9
Ca K	20.3	7.7	5.6

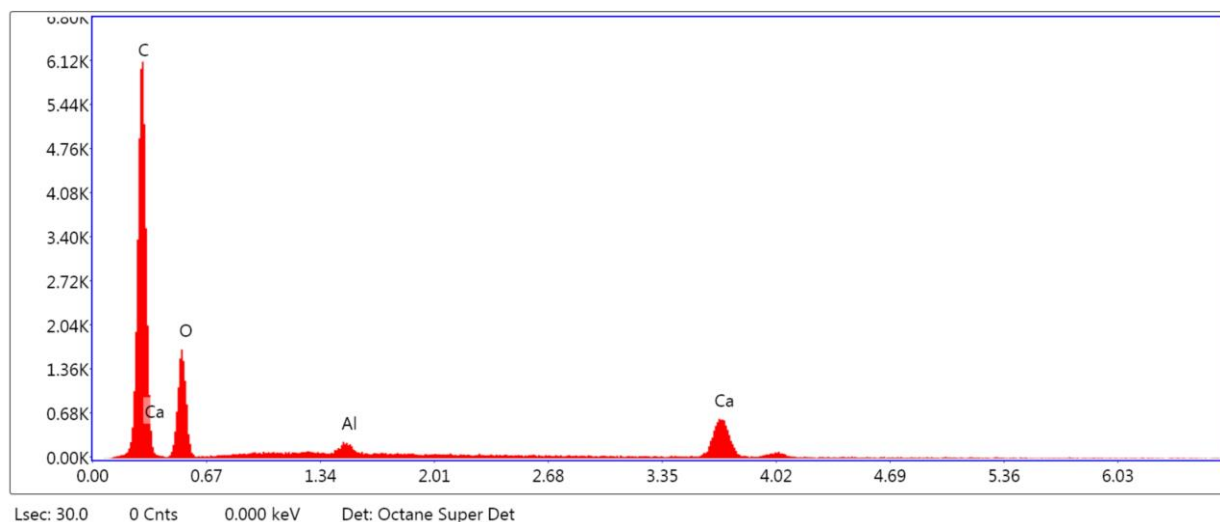


Figure A2.6. EDS results for label 6 (the plate) in Figure 4.2.3.1.

Table A2.6. EDS results for label 6 (the plate) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	53.1	67.0	4.7
O K	26.4	25.1	9.9
Al K	0.8	0.5	11.8
Ca K	19.7	7.4	5.5

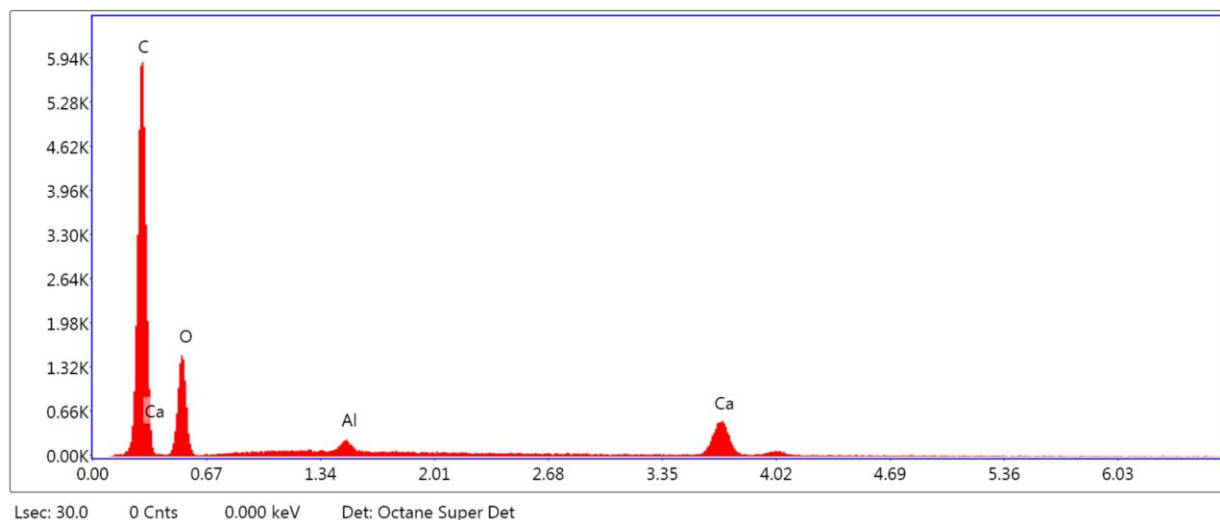


Figure A2.7. EDS results for label 7 (the plate) in Figure 4.2.3.1.

Table A2.7. EDS results for label 7 (the plate) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	55.4	68.7	4.7
O K	25.9	24.2	9.9
Al K	0.9	0.5	11.9
Ca K	17.9	6.6	5.8

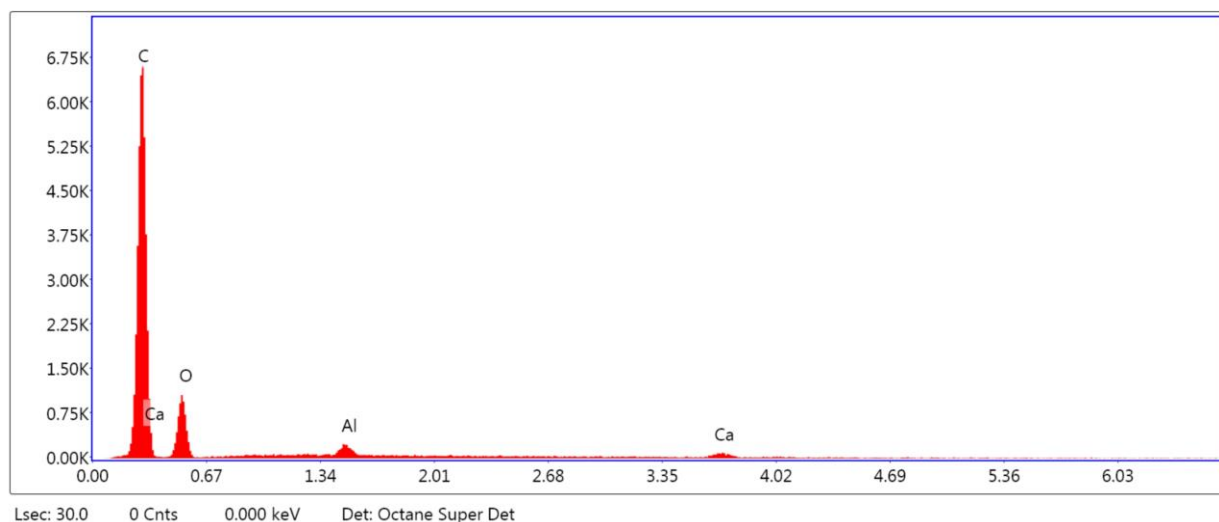


Figure A2.8. EDS results for label 8 (the non-plate place) in Figure 4.2.3.1.

Table A2.8. EDS results for label 8 (the non-plate place) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	74.8	81.4	3.9
O K	20.8	17.0	10.2
Al K	1.1	0.5	11.6
Ca K	3.3	1.1	16.6

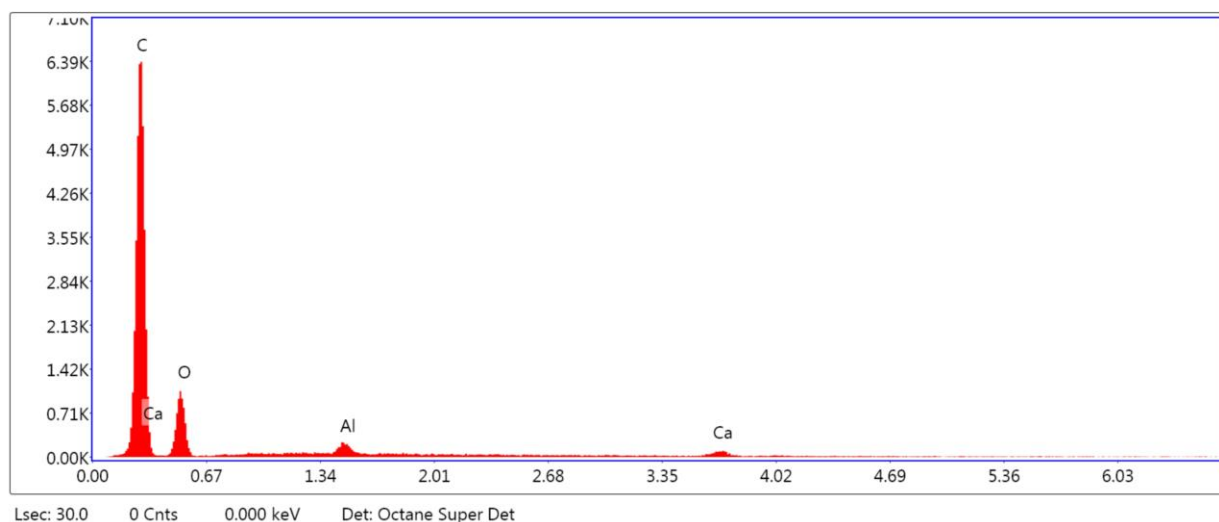


Figure A2.9. EDS results for label 9 (the non-plate place) in Figure 4.2.3.1.

Table A2.9. EDS results for label 9 (the non-plate place) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	73.9	80.7	3.9
O K	21.5	17.6	10.1
Al K	1.1	0.5	11.6
Ca K	3.5	1.2	15.8

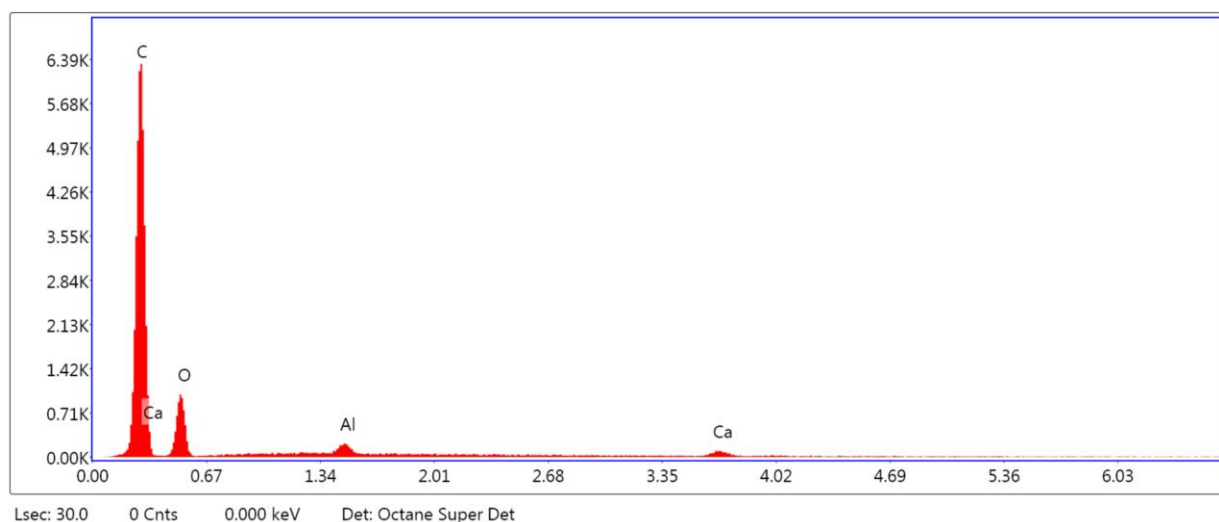


Figure A2.10. EDS results for label 10 (the non-plate place) in Figure 4.2.3.1.

Table A2.10. EDS results for label 10 (the non-plate place) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	74.6	81.3	3.9
O K	20.7	17.0	10.2
Al K	1.0	0.5	11.7
Ca K	3.6	1.2	15.9

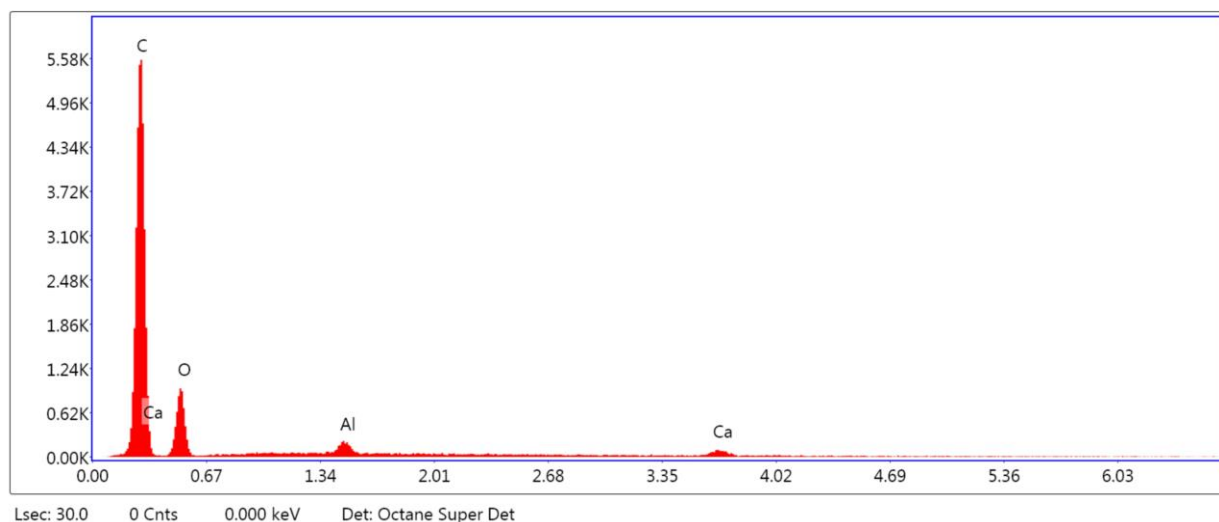


Figure A2.11. EDS results for label 11 (the non-plate place) in Figure 4.2.3.1.

Table A2.11. EDS results for label 11 (the non-plate place) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	73.2	80.3	4.0
O K	21.7	17.9	10.2
Al K	1.2	0.6	11.6
Ca K	3.9	1.3	15.0

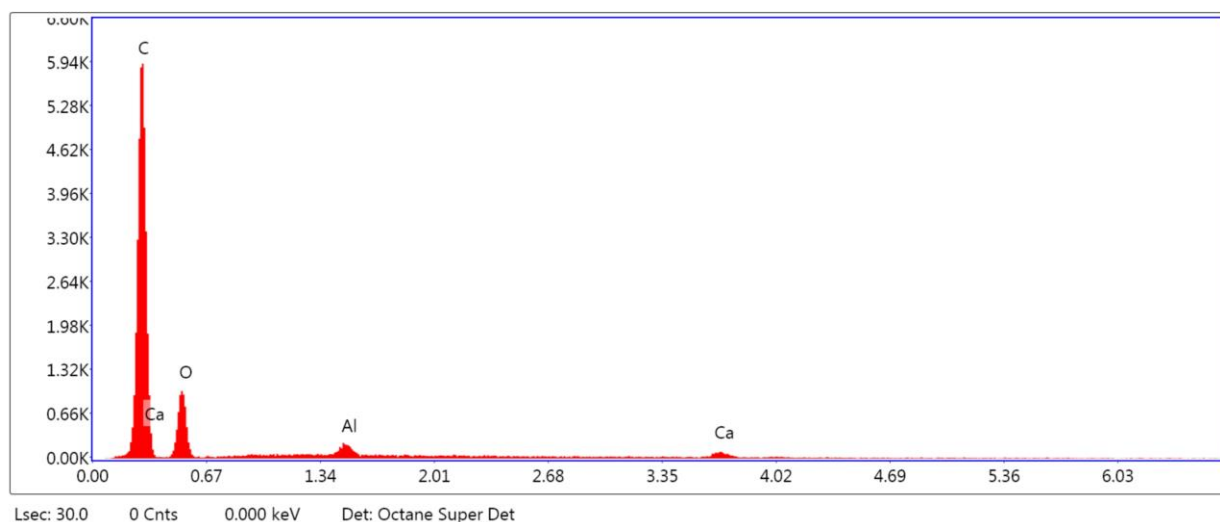


Figure A2.12. EDS results for label 12 (the non-plate place) in Figure 4.2.3.1.

Table A2.12. EDS results for label 12 (the non-plate place) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	73.3	80.2	4.0
O K	22.1	18.1	10.1
Al K	1.1	0.6	11.5
Ca K	3.5	1.2	15.9

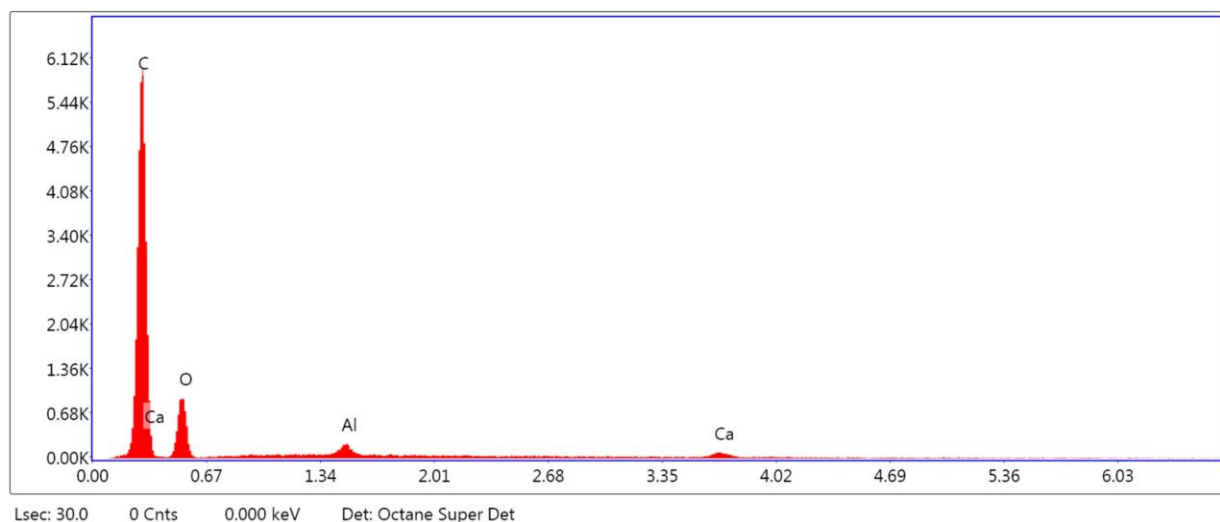


Figure A2.13. EDS results for label 13 (the non-plate place) in Figure 4.2.3.1.

Table A2.13. EDS results for label 13 (the non-plate place) in Figure 4.2.3.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	74.1	80.8	4.0
O K	21.4	17.5	10.2
Al K	1.1	0.5	11.8
Ca K	3.5	1.1	15.6

Appendix III – EDS results for labels in Figure 6.2.2.1

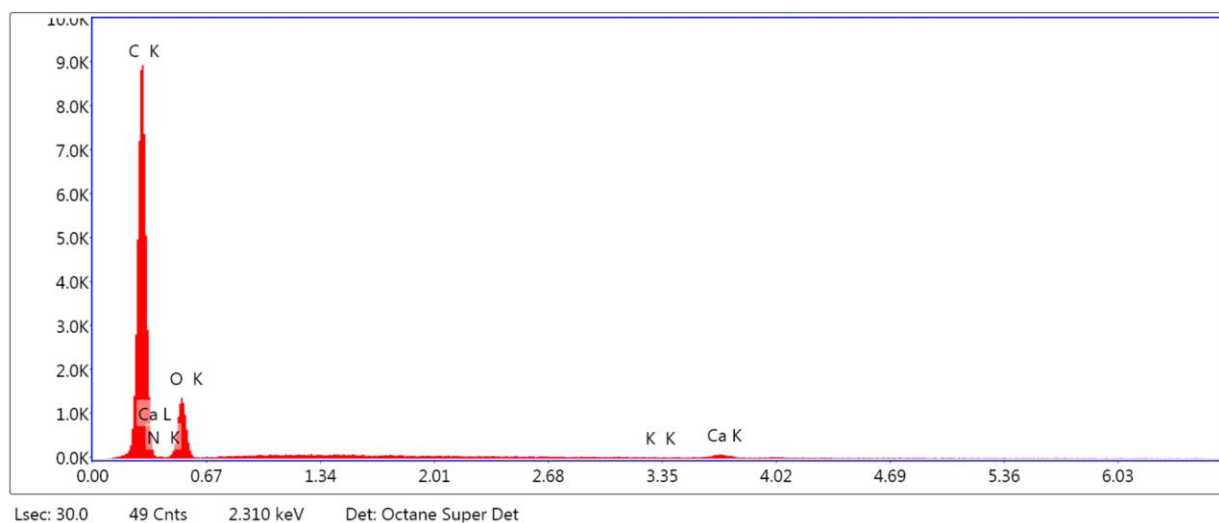


Figure A3.1. EDS results for label 1 (the pit) in Figure 6.2.2.1.

Table A3.1. EDS results for label 1 (the pit) in Figure 6.2.2.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	75.7	81.6	3.5
O K	21.0	17.0	10.0
Ca K	2.7	0.9	18.1

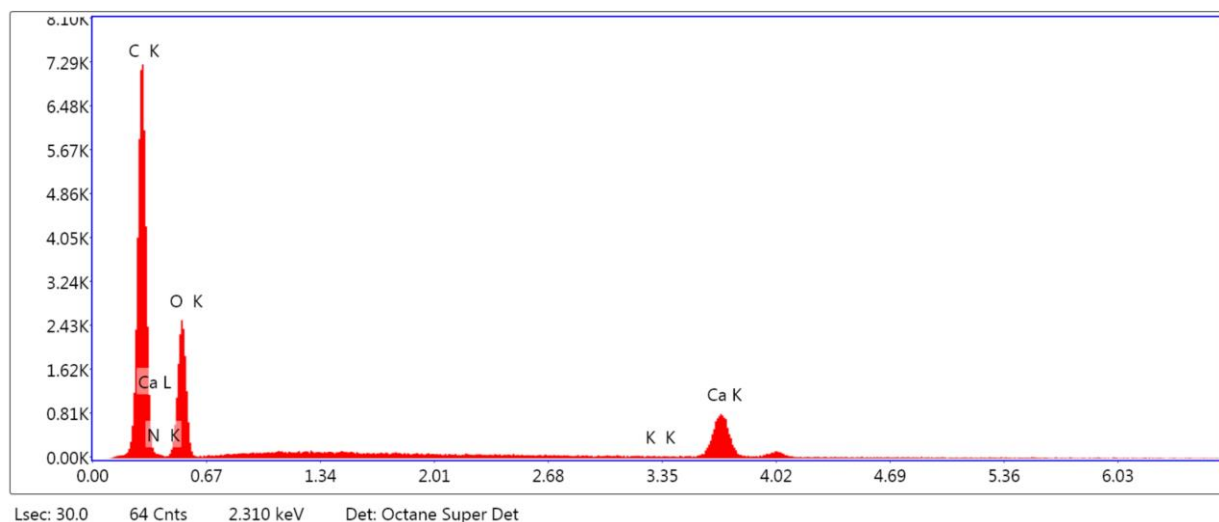


Figure A3.2. EDS results for label 2 (the calcium oxalate plate) in Figure 6.2.2.1.

Table A3.2. EDS results for label 2 (the calcium oxalate plate) in Figure 6.2.2.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	47.4	61.7	4.7
O K	30.3	29.6	9.6
Ca K	22.1	8.6	4.5

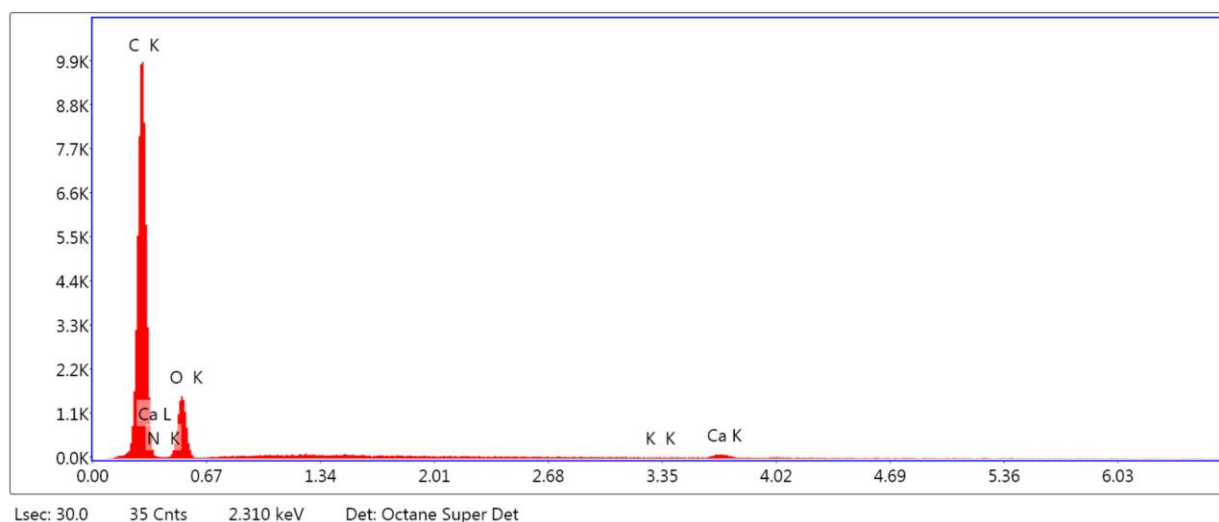


Figure A3.3. EDS results for label 3 (the non-plate place) in Figure 6.2.2.1.

Table A3.3. EDS results for label 3 (the non-plate place) in Figure 6.2.2.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	75.4	81.4	3.5
O K	21.4	17.3	9.6
Ca K	2.5	0.8	16.3

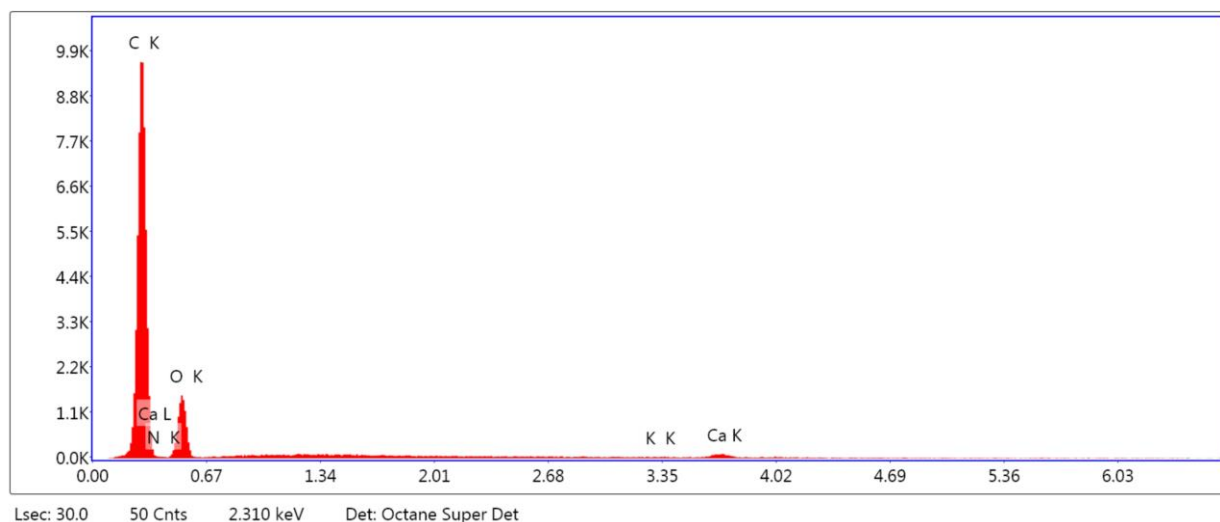


Figure A3.4. EDS results for label 4 (the non-plate place) in Figure 6.2.2.1.

Table A3.4. EDS results for label 4 (the non-plate place) in Figure 6.2.2.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	75.6	81.7	3.5
O K	21.1	17.1	9.9
Ca K	2.9	0.9	14.3

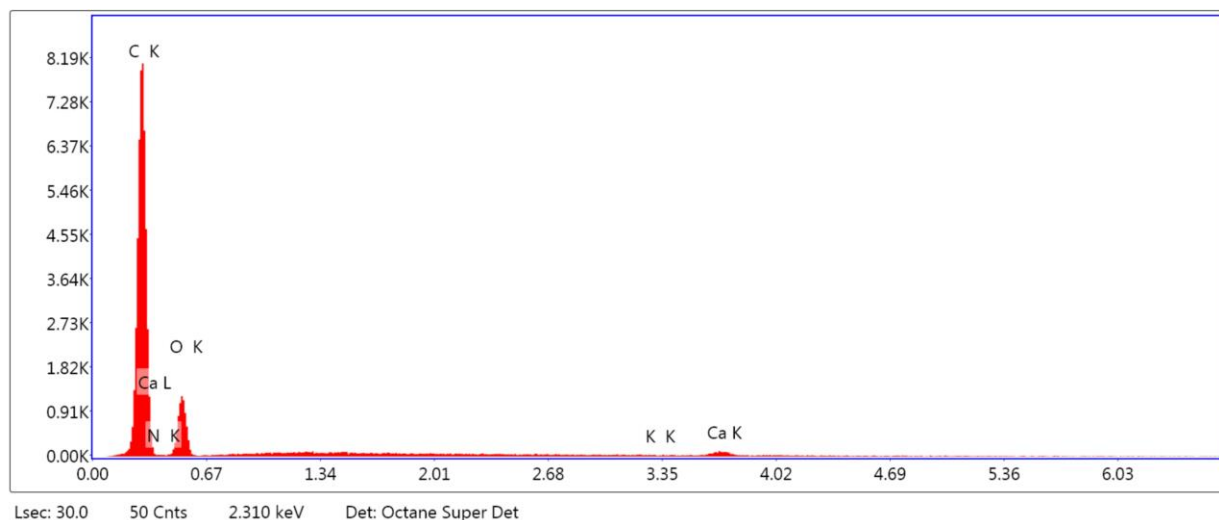


Figure A3.5. EDS results for label 5 (the pit) in Figure 6.2.2.1.

Table A3.5. EDS results for label 5 (the pit) in Figure 6.2.2.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	75.8	82.1	3.5
O K	20.5	16.7	10.1
Ca K	3.5	1.1	15.5

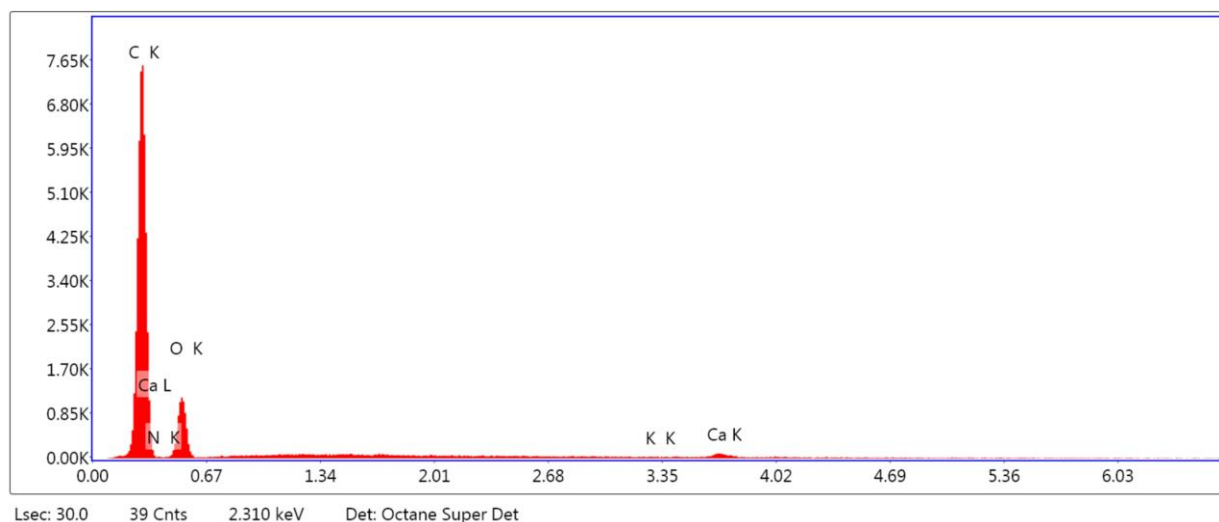


Figure A3.6. EDS results for label 6 (the pit) in Figure 6.2.2.1.

Table A3.6. EDS results for label 6 (the pit) in Figure 6.2.2.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	75.7	81.9	3.5
O K	21.0	17.0	10.1
Ca K	3.1	1.0	17.3

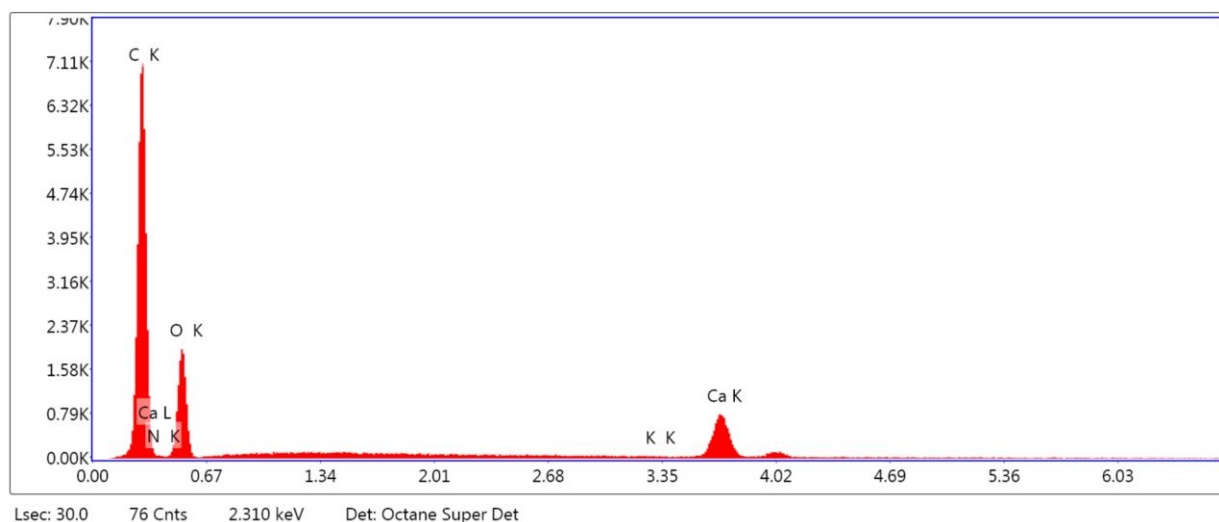


Figure A3.7. EDS results for label 7 (the remainder of the calcium oxalate plate) in Figure 6.2.2.1.

Table A3.7. EDS results for label 7 (the remainder of the calcium oxalate plate) in Figure 6.2.2.1. The letter “K” was from the raw data and it indicated the energy-level shell.

Element	Weight %	Atomic %	Error %
C K	49.6	64.6	4.6
O K	26.8	26.2	9.8
Ca K	23.4	9.1	4.6

Appendix IV – Statistical analysis

F-test was used to compare the variance of diameters of the manually extracted fibers (variance $\approx 77.3 \mu\text{m}^2$) and the chemically treated (at room temperature) manually extracted fibers (variance $\approx 4.3 \mu\text{m}^2$). The p-value was $1.268 \times 10^{-7} < 0.05$. Therefore, the variance of diameters of the manually extracted fibers was statistically larger than that of the chemically treated (at room temperature) manually extracted fibers.

The variance of diameters of the manually extracted fibers (variance $\approx 77.3 \mu\text{m}^2$) and the chemically treated (at $70 - 80^\circ\text{C}$) manually extracted fibers (variance $\approx 22.9 \mu\text{m}^2$) were compared. The p-value was $0.01552 < 0.05$. Therefore, the variance of diameters of the manually extracted fibers was statistically larger than that of the chemically treated (at $70 - 80^\circ\text{C}$) manually extracted fibers.

The variance of diameters of the chemically treated (at room temperature) manually extracted fibers (variance $\approx 4.3 \mu\text{m}^2$) and the chemically treated (at $70 - 80^\circ\text{C}$) manually extracted fibers (variance $\approx 22.9 \mu\text{m}^2$) were compared. The p-value was $0.0009395 < 0.05$. Therefore, the variance of diameters of the chemically treated (at room temperature) manually extracted fibers was statistically less than that of the chemically treated (at $70 - 80^\circ\text{C}$) manually extracted fibers.

Welch two sample t-test was used to compare the mean diameter of the manually extracted fibers (mean $\pm$ SD $\approx 49.4 \pm 8.8 \mu\text{m}$) and the chemically treated (at room temperature) manually extracted fibers (mean $\pm$ SD $\approx 30.6 \pm 2.1 \mu\text{m}$). The p-value was $2.028 \times 10^{-8} < 0.05$. Therefore, the diameters of the manually extracted fibers had a statistically larger mean than that of the chemically treated (at room temperature) manually extracted fibers.

The mean diameter of the manually extracted fibers (mean $\pm$ SD $\approx 49.4 \pm 8.8 \mu\text{m}$) and the chemically treated (at 70 – 80°C) manually extracted fibers (mean $\pm$ SD $\approx 29.5 \pm 4.8 \mu\text{m}$) were compared. The p-value was $4.349 \times 10^{-9} < 0.05$. Therefore, the diameters of the manually extracted fibers had a statistically larger mean than that of the chemically treated (at 70 – 80°C) manually extracted fibers.

The mean diameter of the chemically treated (at room temperature) manually extracted fibers (mean $\pm$ SD $\approx 30.6 \pm 2.1 \mu\text{m}$) and the chemically treated (at 70 – 80°C) manually extracted fibers (mean $\pm$ SD $\approx 29.5 \pm 4.8 \mu\text{m}$) were compared. The p-value was $0.4362 > 0.05$. Therefore, the mean diameters of the chemically treated (at room temperature) manually extracted fibers and the chemically treated (at 70 – 80°C) manually extracted fibers were not statistically different.

Note that the diameters of nodes were not included in the data (F-test and Welch two sample t-test).