Characterization and analysis of the molecular weight of corn corbs microcrystalline cellulose (MCC) fiber using mass-spectrometry methods

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Characterization and analysis of the molecular weight of corn corbs microcrystalline cellulose (MCC) fiber using mass-spectrometry methods

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Abstract. Microcrystalline cellulose(MCC) was isolated from corncobs waste. It was activated by NaOH solution with varied concentration in range from 4%, 6%, 8%, 10%, 12%, 14% and 17%. Characterization of physicochemical properties of microcrystalline cellulose (MCC) molecular weight was conducted by using Mass Spectrofotometer (MS).Preconditioning of MS used 20 µl injection volume, 10 µl concentration, acethonitrile: ultra water (50:50) as eluent. The result showed that NaOHconcentration affect to the type of oligosaccharides fragmentationwith an increased abundance. MS spectra for NaOH 4% showed four major peaks at 834 m/z, 623 m/z, 425 m/z, 383 m/z that indicated 2-5 glucose polymers respectively, with an increased abundance withadditional Na^+ and K^+ ions.NaOH8%, 12%, 14%, 17% indicated the type of oligosaccharide fragmentation with equal three major peaks at 827 m/z, 623 m/z, 425 m /z. The fragmentatition type of NaOH 10% and avicel standard pH 102, indicated 2-5 glucose polymers respectively,with an increased abundance with additional Na^+ and K^+ ions, there are peak at 827 m/z and 425 m/z, and there is no peak at 623 m/z because there are no Na^* and K^+ ions.

1. Introduction

Cellulose is an interesting and sought of material due to current demands for green chemistry, more environmentally friendly resources and renewable raw material. The use of cellulose is therefore highly atractive because of it being renewable, biodegradable and non-toxic. Cellulose is a homopolysaccharide consisting of unbranched units of D-glucose (anhydroglucose) connected to 1 and 4 C atoms with β-glycosidic binding (β-1,4 glycosidic bond) [1]. Microcrystalline Cellulose (MCC) one of the cellulose derivative that can be obstained by enzimatik hydrolysis [2] extracted by acid hydrolysis with H_2SO_4, hydroclorid acid [3] [4]. MCC is a purified, partiallyde-polimeryzed form of cellulose occurring as a fine, freeflowing crystalline powder. MCC has been used for many years in different industries like pharmaceuticals, cosmetics, plastic, food etc [4]. Cellulose has four different crystalline forms named cellulose I-IV.The crystal structure of cellulose I in native cellulose can be converted to cellulose II by dissolution and regeneration or merserization [5].

Polymer characterization is generally performed to evaluate the quality of the synthesis or substitute product one of which is the determination of molecular weight. Molecule weight affects the cellulose dissolution process in the IONCELL-P process using 1-etil-3-methylimidazolium asetat ([emim] OAc) and water solvent. Degree of the crystanillity, accessibility and reactivity of the cellulose chains are important properties of the cellulose [6]. Determination of molecular weight (BM), average molecular weight (Mw) and polydispersity index (D) depending on biomass source,

solvent usage pretreatment conditions and isolation methods. Cellulose has a high molecular weight (MW), with polymerization (DP) levels up to 20,000.The molecular mass value describes the polymeric molecular size distribution, an important variable determining the polymer's chemical properties. High molecular weight polymers have stronger properties. In other polymerizations, polymers with very low molecular weight distributions can be obtained [7].

The properties of polymers, such as viscosity depend on molecular weight and molecular weight distribution. Therefore, it is important to determine the average molecular weight associated with the distribution of specific molecular weights. The polymerization reaction produces a weight distribution and a molecular shape. The molecular weight of the polymer can be expressed as the average molecular weight, and polydispersity/Molecular Weight Distribution (MWD). The polydispers index represents the quality of degradation. Molecular weight is highly influential on the physicochemical properties of cellulosic material [8] [9]. Some methods for determining these parameters are Gel Permeation Chromatography (GPC) and Mass Spectrometry (MALDI TOF-MS) and Size-Exclusion Chromatography, SEC is a technique of determining the molecular weight of polymers with a short time. The molecular weight can be expressed by the average molecular weight (Mn), average molecular weight (Mw), average molecular weight (Mz), average molecular weight of viscosity (Mv) [10] [11] [12]. Aplication of size exclusion chromatography (SEC) combinated with electrospray ionization mass spectrometry (ESI-MS) for the determination of accurate moleculer weight distribution corrected for chromatography brand broadening [13].

LC-MS-technieque is used to determine the molecular weight of microcrystalline cellulose (MCC). Liquid chromatography (LC) is eminently suistable for separating soluble polymers. In Mass-Spectrometry (MS) the molecules in a sample are first ionized, than separated according to their mass to charge rasio (m/z) and finally detected. MS provides accurate information on the molecular weight of the separated and detected ions [10].

2. Research Method

2.1. Material, Chemicals and Tools

Corncob from Milango Village, Pohuwato District, Gorontalo Province, Indonesia. Corncob waste was cut into small pieces ± 2cm, washed with aquadest and in oven temperature 60^0C-70^0C for 24 hours. The dried sample is blended and in the shaked to obtain corncob powder particles 180 micron by size [4].The reagents were of analytical grades, NaOH (reagent grade, 98%), HCl (ACS reagent, 37%), H_2O_2 (technical grade) were supplied by Sigma-Aldrich, $K_2Cr_2O_7$, H_2SO_4, Avicel 102 (technical grade) a commercial brand of MCC, Ethanol (95% pa), Tholuena (95% pa) and distilled water used as solvent. Instruments using Shaker Digital ASTM E11 180 micron, pH meter (Thermo SCIENFIC ORION), LC-MS waters UPLC MS G-2 QTQF. System : ESI (Electrospray Ionization). Instrument condition of volume injection 20 µl, concentration 10 µl, acethonitrile:ultra water (50:50) as eluent.

2.2. Method

Cellulose isolation, purification of α-cellulose and the preparation of microcrystalline cellulose (MCC) is a modified procedure [4] that is : dewaxing stage: 50 gr of 180micron corncob powder was soxhleted in ethanol :tholuene (2:1) for 4hours. Sample was filtered and washed until neutral pH, then it was oven-dried at 60^0C for 8 hours. De-hemicellulose stage: 10 gr of 180 micron corncob powder was dissolved into 200 mL of 4%, 6%, 8%,10%, 12%, 14% and 17% NaOH solution. The mixture was heated on hot plate at 100^0C for ± 2 hours. Then it was filtered and washed until neutral pH, oven-dried at 60^0C. De-lignification and bleaching stage using H_2O_2 4% for ± 1 hour at room temperature. Then sample was filtered and washed until neutralpH. It was oven-dried at 60^0C for 8 hours. Further purification of α-cellulose was done by 5 gr of cellulose dissolved in 500 ml of 17.5% NaOH at 80^0C for 30 min. Furthermore the precipitate was filtered and washed until neutral pH, and oven-dried at 60^0C for 8 hours. The preparation of microcrystalline cellulose (MCC) that is: α-cellulose product was

hydrolysed with 0.1N HCl ratio 1: 2 and refluxed at 80^0C for ± 2 hours. The precipitate was filtered and washed with aquadest until neutral pH and oven-dried at 60^0C for 8 hours.

Microcrystalline Cellulose (MCC) product characterized by molecular weight using MS Analysis was performed using waters MS G-2 QTQF. Full scan mode from m/z 100 to 1200 was performed with a source temperature at 140^0C. Solvent was Acethonitrile 50%.

3. Result and discusion

3.1. Effect of NaOH concentration on α-cellulose acquisition

Degradation of the cellulose under alkalline condition starts from the reducing and group of the cellulose at moderate temperature 80-100^0C [6]. Pretreatment process with NaOH was to lysis and reduce lignin and hemicelullose, to break the crystal structure of celullose and to improve the porosity of sample [17]. The result showed that conversion ratio of cellulose is high than enzymatic hydrolysis [19]. Produce isolation of cellulosewith heating NaOH 4%, 6%, 8%, 10%, 12%, 14%, 17% and preparation of microcrystallinecellulose (MCC) at Figure 1.

(a) (b) (c) (d)

Figure 1.(a) produce of cellulose, (b) purification of α-cellulose, (c-d) preparation MCC

Here is the curve of purified α-cellulose content in variations of NaOH concentration of 4%, 6%, 8%, 10%, 12%, 14% and 17%.

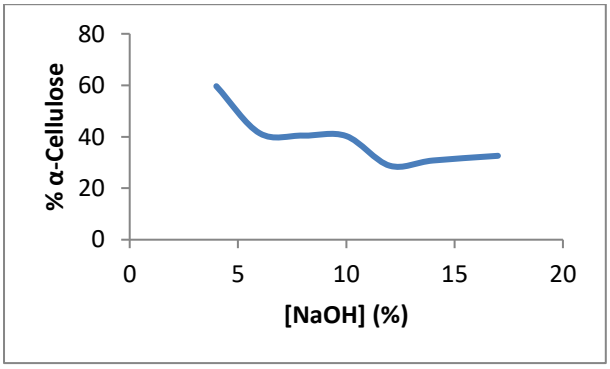

Figure 2. Effect of NaOH concentration on *α*-cellulose acquisition

NaOH 4% gave higest α-selulosa content i.e 60% (figure 2). When NaOH concentration was higher, the α-selulosa content decreased. It is because the bonds of celullose chain are not tightly , therefore celullose molecules had dispersed freely in NaOH solutions. This allows celullose to pass when filtering and washing.[15]The addition of NaOH 5% (w/w) on *Chlorella vulgaris* at 50^0C for 24 hour gave 9,8% of carbohydrate solubility compared with no NaOH added.When NaOH concentration

that add to *Scenedesmus sp* is higher, the solubility of carbohydrate increased. In addition, NaOH cause autohydrolysis reaction that make microorganism release hyrdrolyze enzyme. Celullose content of biomass was expected to increase in pretreatment process [18].

3.2. Effect of NaOH Concentration on Fragmentation Type of Oligosaccharida

Polymers are materials with chains of varying length, and each chain consists of monomer residues that affect its properties and thus require the characterization of several parameters [2]. There is influence of NaOH concentration to fragmentation type. If NaOH concentration increased, the spectra showed an increased abundance. The cellulose II crystallites in regenerated celluloses increase in size to the longitudinal direction by the alkaline treatment and acid hydrolysis. There are increase cristallinity size with alkaline treatment in 20% NaOH and acid hydrolysis at 105 °C[12]. Breakdown of celullose structure will make celullose lysis more easier. Simply sugar was the product of lysis, then will be fermented by certain microorganisms [16]. The following is a type of oligosaccharide fragment in several variations of NaOH concentration.The following types of oligosaccharide fragmentation in 4% NaOH;

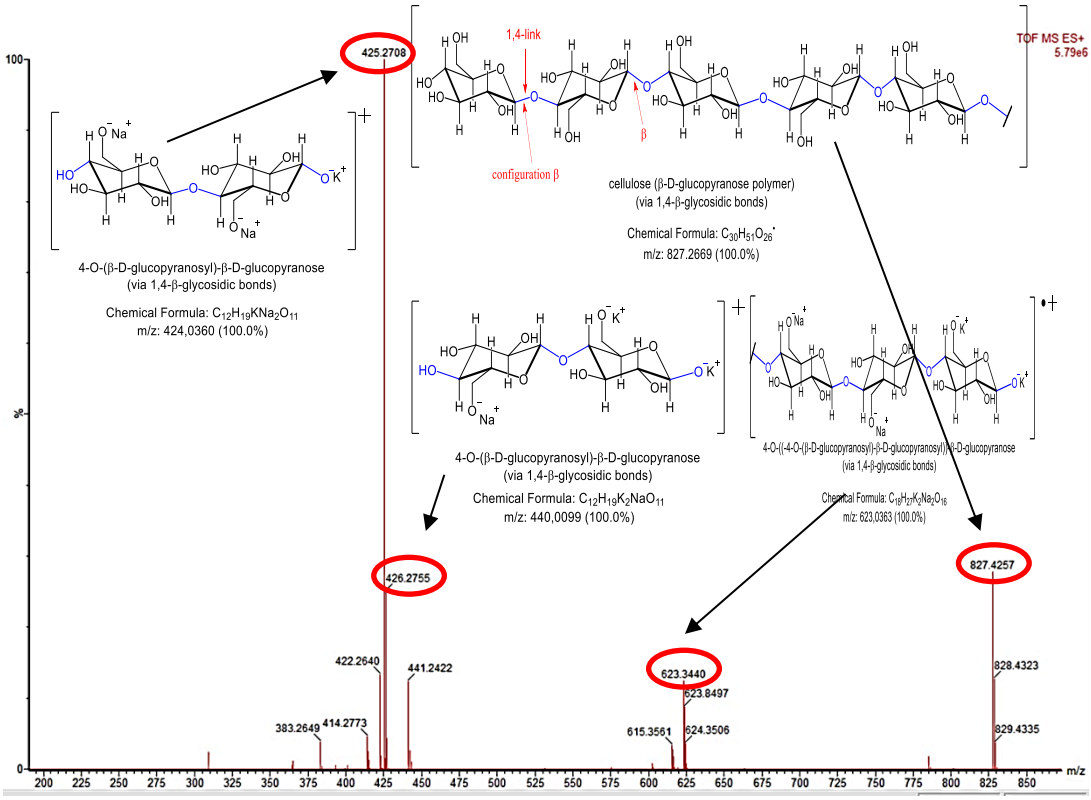

Figure 3. Fragmentation type of oligosaccharida with NaOH 4%

Based on MS spectra of NaOH 4%showed that corncob samples were olighoshacaride. Oligosaccharides consist of short chains of monosaccharide units combined by covalent bonds, including disaccharides having two monosaccharide units, eg sucrose or sugar cane consisting of 6-carbon D-glucose sugar and D-fructose.Most oligosaccharides have three or more non-free units but are combined as polypeptide side chains in glycoproteins and proteoglycans [14]. The ES-MS spectra of 4%NaOHshow peak of ion $[M]^+$ at 827,4257 m/z, andappropriate with molecular mass (Mr) of oligosccharide ($C_{30}H_{51}O_{26}^{\bullet}$) 827,2669 g/mol. NaOH 4% gave higher α-selulosa content ii.e 60% (Fig.2)This indicated the presence 5 polimer of glucose. The determination of the molecular weight of

the lignocellulose in the original biomass depends on the source of the bioresources used and the procedure and purification used.The average weight (Mn), average molecular weight (Mw) and polydispersity index (D) all vary greatly depending on biomass sources, pre-treatments and methods [6][7]. Here is (Figure. 4) the fragmentation of oligosaccharide:

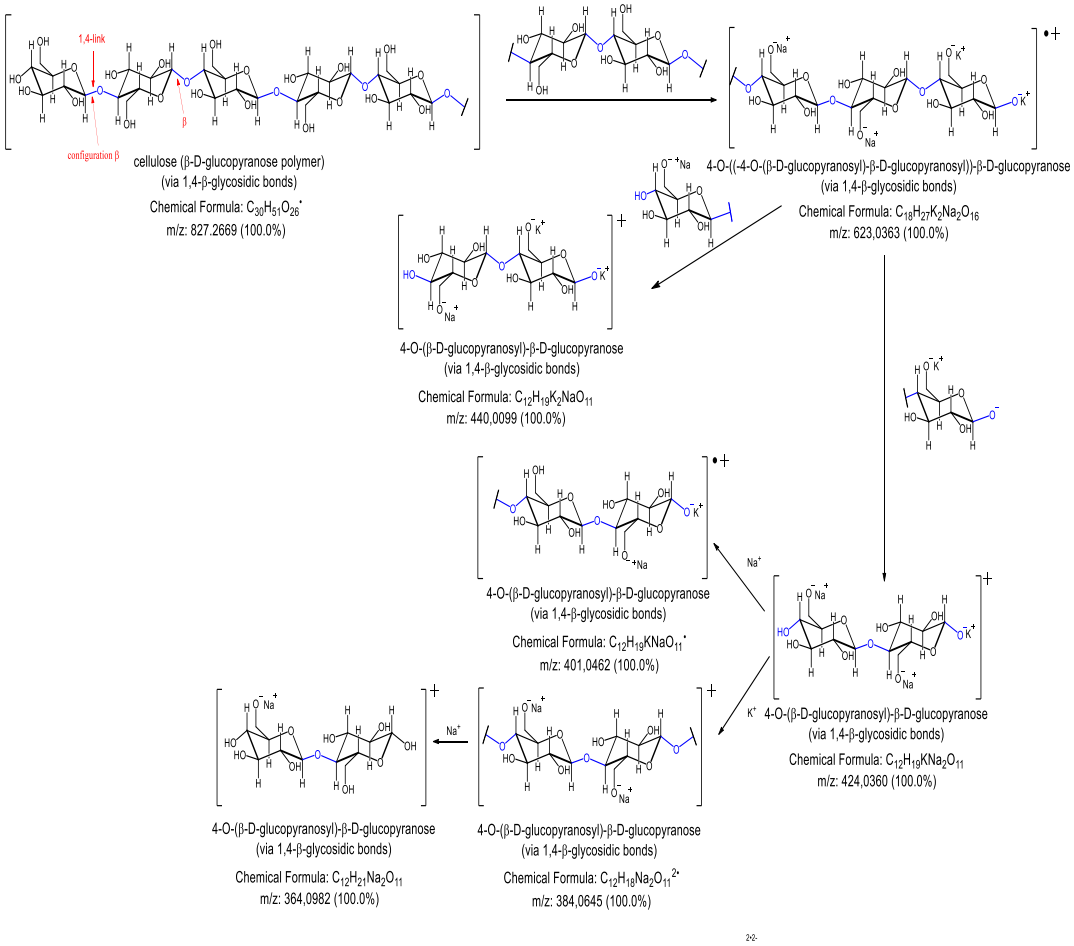

Figure 4. Oligosaccharide fragmentation

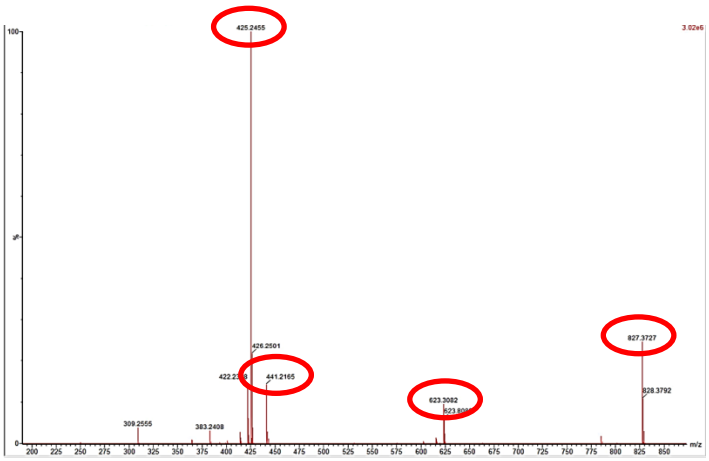

Figure5 . Fragmentation type of oligosaccharida with NaOH 8%

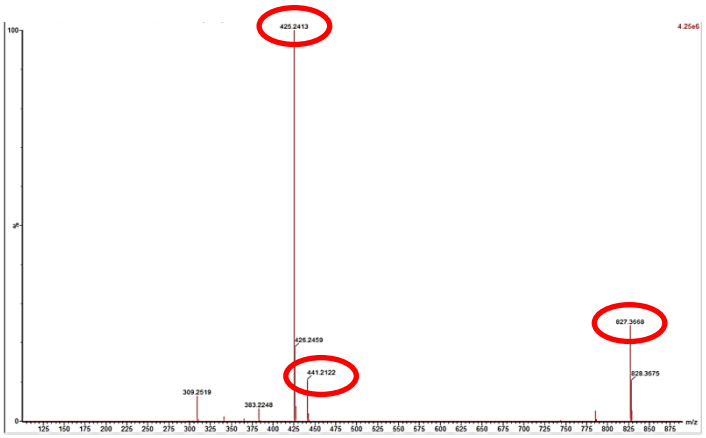

Figure6 . Fragmentation type of oligosakarida with NaOH 10%

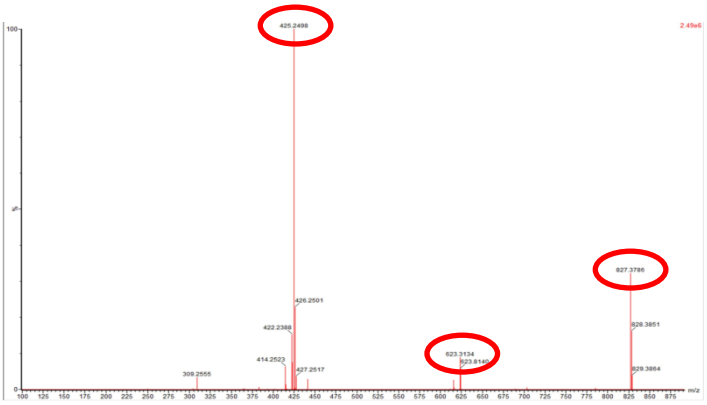

Figure7. Fragmentation type of oligosakarida with NaOH 12%

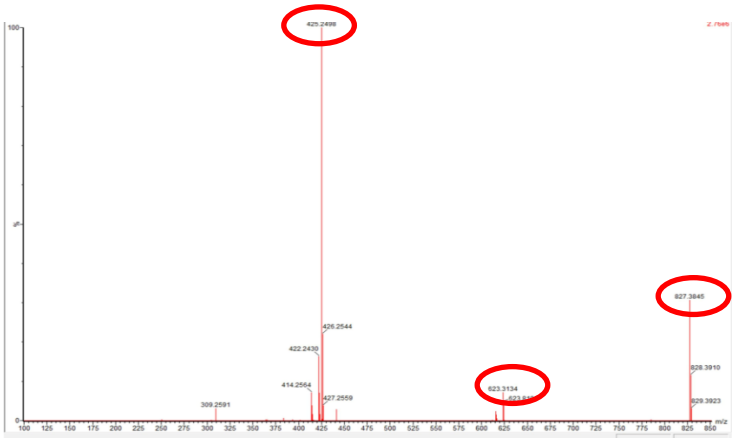

Figure8 . Fragmentation type of oligosakarida with NaOH 14%

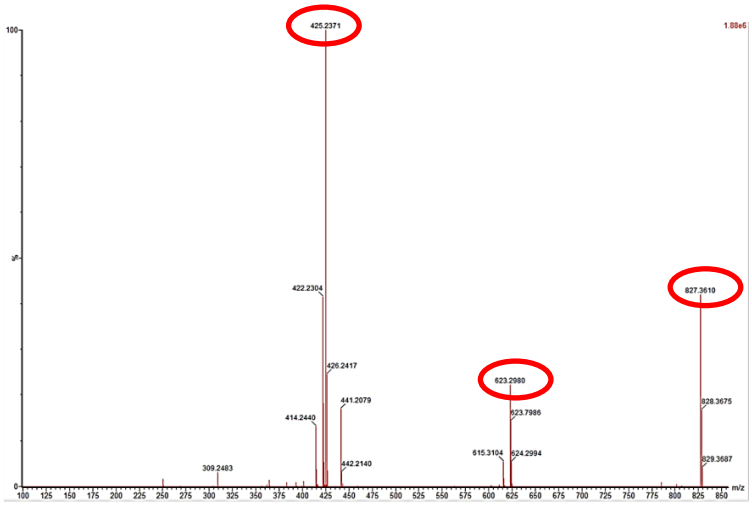

Figure 9 . Fragmentation type of oligosakarida with NaOH 17%

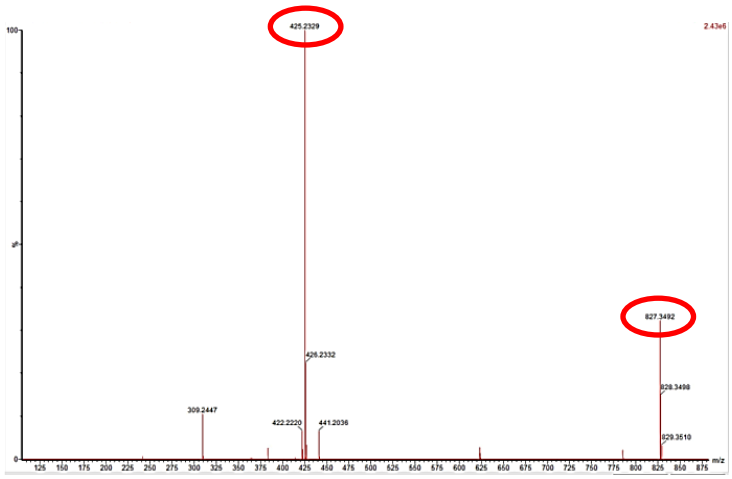

Figure10. Standard Avicel 102

Based on MS spectra of NaOH 8%,10%,12%,14%,17%showed that corncob samples were olighoshacaride. Oligosaccharides consist of short chains of monosaccharide units combined by covalent bonds, including disaccharides having two monosaccharide units, eg sucrose or sugar cane consisting of 6-carbon D-glucose sugar and D-fructose.Most oligosaccharides have three or more non-free units but are combined as polypeptide side chains in glycoproteins and proteoglycans [14]. The ES-MS spectra of 8%,10%,12%,14%,17%NaOHshow peak of ion $[M]^+$ at 827,4257 m/z, andappropriate with molecule mass (Mr) of oligosccharide ($C_{30}H_{51}O_{26}^{\bullet}$) 827,2669 g/mol. This indicated the presence 5 polimer of glucose. High radiation on celluoce biomssa will increase surface area, reduce polymerization degree and celullose crystallinity, hydrolize hemicellulose and cause lignin de polimerizaation. When surface area is high, the glucose from celullose increased [20].

Spectraof ion $[M]^+$at 623,3440 m/z indicated the presence 3 glucose with additionalion–ion $2Na^+$and $2K^+$ion, with molecular mass (Mr) 623,0363 g/mol. There are 3 possibilities i.eNa^+ and $2K^+$ ions from ultra water still have mineral contaminant, Na^+ and K^+ ions as intraseluler ions in plant that carried away during extraction of cellulose with NaOH and $K_2Cr_2O_7$ as activator. Spectra peak $[M+H]^+$ ion at 441,2422 m/z show 2 glucose with additional $2K^+$ and Na^+ ions, that appropriate with molecular formula Mr440,0099 g/mol and molecular formula i.e$C_{12}H_{19}K_2NaO_{11}$. Spectra peak $[M+H]^+$ion at 425,2708 m/z show 2 glucose with additional K^+ dan 2Na ions, that appropriate with molecular formula Mr424,0360 g/mol and molecular formula i.e$C_{12}H_{19}KNa_2O_{11}$.

Based on the result, compound inside concorbi.ecellobiose oligosaccharide. Cellobiose is non reducing glucose that composite matrix of cellulose [14].There is elimination reaction at β-alkoxy group, if NaOH concentration increase8%,10%,12%,14%,17%. This is causing the dissolved monosaccharide units and shortening the polysaccharide chain [1].The MS spectra of Avicel standard pH 102 indicate the type of oligosaccharide fragmentation with same two main peaks i.e at 827 m/z, that indicated the presence of 5 Glucose polymers. The absence of spectra at 623 m/z peak showed that there is no additional Na^+ and K^+ in 3 glucose polymer.

Table 1. Interpretation oligosaccharide fragmentation with NaOH

No Peak	m/z						Structure
	4%	8%	10%	12%	14%	17%	
1	827,4257	827,3727	827,3668	827,3786	827,3845	827,3610	[G5]
2	623,3440	623,3082	-	623.3134	623.3134	623.2980	[G3 + 2K + 2Na]
3	441,2422	441,2165	441,2122	-	-	441.2079	[G2 + 2K + Na]
4	425,2708	425,2955	425,2413	425,2498	425,2498	425,2371	[G2 + K + 2Na]

4. Conclusions

If NaOHconcentration increased, this is affect the type of fragmentation with increasing abundance. NaOH 4% shows the four main peaks of 834 m/z, 623 m/z, 425 m/z, 383 m/z respectively indicating the presence of 2 - 5 glucose polymers with an increased abundance i.e the addition of $2Na^+$ and $2K^+$. The concentration of NaOH 8%, 12%, 17% showed the type of oligosaccharide fragmentation with the same three main peak at 827 m/z, 623 m/z, 425 m/z. For NaOH10%, the oligosaccharide fragmentation type indicated the presence of 2 - 5 Glucose polymers with an increased abundance of the addition of $2Na^+$ and K^+ ions, with absence peak at 623 m/z.

Acknowledgements

Based on the research and the desired results for subsequent researchers to perform the measurement and analysis.

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