

Bacterial Cellulose and Its Phosphonic Dichloride for Efficient Removal of Metal Ions

Sherif M. A. S. Keshk^{1,2,*}, Mohamed S. Omar^{3,4}

¹King Khalid University, Faculty of Science, Chemistry Department, P.O. Box 9004, Abha 61413- Saudi Arabia

²Ain Shams University, Institute of Environmental Studies and Research, Basic Science Department, Abbassia, Cairo 11566, Egypt

³Benha University, Faculty of Science, Chemistry Department, Qalyubia, Egypt

⁴Taif University, College of Pharmacy, Pharmacology and Toxicology Department, Taif, Saudi Arabia

Abstract Bacterial cellulose (BC) in comparison with cotton stalks (CS) and their phosphonic dichloride derivatives for efficient removal of metal ions were investigated. BC and its phosphonic dichloride have the highest efficiency toward metal ion uptake, due to its low degree of polymerization and the largest surface area. FT-IR results showed two bands at 1198 cm^{-1} and 960 cm^{-1} that characteristic to C-O-P bond. Furthermore, the fragments obtained from mass spectrum was characteristic to six carbon sugar mono phosphonic dichloride for both BC and CS. X-ray diffractogram confirmed the presence of phosphorous group at $2\theta = 30^\circ$ that corresponding to (211) plane.

Keywords Bacterial cellulose, Cotton stalks, Phosphonic dichloride, Degree of polymerization

1. Introduction

The consumption of disposable materials has increased and hence, the load on the environment has increased dramatically. Consequently, the demand for bio-based materials with renewable, low-cost and sustainable properties has increased. Cellulose-based materials can potentially provide products to meet this demand. Annual cellulose synthesis by plants is close to 3×10^{12} tons (Klemm et al, 1998). Plants contain approximately 33% cellulose, whereas, wood contains around 50% and cotton contains 90%. Cotton and wood are the major resources for all cellulose products such as paper, textiles, construction materials, and cardboard, in addition to cellulose derivatives such as cellophane, rayon, and cellulose acetate (Colvin, 1980). Unlike the cellulose from wood pulp, cellulose produced by an *Acetobacter* strain is devoid of other contaminating polysaccharides and its isolation and purification are relatively simple, not requiring energy- or chemical-intensive processes. Furthermore, environmental problems due to by-products of wood pulping given an added impetus to study unexplored sources of cellulose (Keshk, 2014 a, b). Bacterial cellulose (BC) generated as a never-dried membrane from static culture in a nearly pure form that contains 99.1wt% water of which 0.3wt% is bound and 98.8wt% is free of water (Brown et al., 1976). It has more than 200 times greater surface area than isolated

softwood cellulose and has a tensile strength similar to that of steel (Delmer & Yehudit, 1995). BC is excellent adsorbents for organic dyes, due to their fibrous structure and high hydrophilic sorption capacity (Delmer & Yehudit, 1995). Cellulose can be chemically modified to yield derivatives which are used widely in different industrial sectors in addition to conventional applications. As an example, cellulose was used as a raw material in the production of regenerated fibres and films in addition to cellulose derivatives (Klemm et al, 1998). Derivatives are further used as coatings, laminations, optical films and absorbents. Additionally, cellulose derivatives can be found as additives in building materials and also in pharmaceutical, food and cosmetic products (Klemm et al, 1998; Keshk & Hajia, 2011). The reactivity and availability of the hydroxyl groups of BC have been investigated via chemical reactions as well as the subsequent characterization of the derivatives obtained (Keshk & Nada, 2003; Keshk, 2008, 2011, 2014a). BC is more reactive than other natural cellulose towered homogenous and heterogeneous reactions owing to increased number of the hydroxyl groups in BC chains in addition to its lower degree of polymerization than those of natural cellulose (Keshk, 2008; Keshk & Nada, 2003). The removal of heavy metal ions from aquatic systems is the subject of extensive technological research and recovering processes. Various methods have been reported for the treatment of aqueous streams contaminated with heavy metals, among them polymers (Rivas, 1998; Zhou et al., 2004; Okieimen et al., 2005; Keshk & Nada, 2008) amorphous silica (Padilha et al., 1999; Goswami & Singh, 2002), clays (Mercier & Detellier, 1995; Mercier & Pinnavaia, 1998, Hasine et al., 2008) and zeolites (Ahmed

* Corresponding author:

keshksherif@gmail.com (Sherif M. A. S. Keshk)

Published online at <http://journal.sapub.org/ajps>

Copyright © 2014 Scientific & Academic Publishing. All Rights Reserved

et al., 1998; Erdem et al., 2004). Nevertheless, these materials have shown several problems like low mechanical and thermal stability, weak chemical labeling with the metals, poor removal efficiency, high cost etc. The effective and economic methods are based on adsorption process, so the synthesis of adsorbents for the removal of toxic heavy metal ions from waste water is a continuing research objective of environmental pollution control processes. In this work, the efficiencies of BC and CS to remove heavy metal ions (Cr^{3+} , Fe^{3+} , Cu^{2+} , Zn^{2+} and Pb^{2+}) from aquatic systems in comparison with phosphonic dichloride derivatives of both BC and CS will be evaluated. Furthermore the chemical structure of phosphonated cellulose will be discussed using FT-IR, mass spectra and X-ray diffraction.

2. Materials & Methods

The raw materials used in this study were CS (DP = 1250) and BC (DP 890). American type culture collection (ATCC) is the supplier of the *Gluconacetobacter xylinus*. The chemicals used throughout this work were purchased from Sigma and Aldrich Chemical Co.

2.1. Culture Media and Conditions

The culture medium is namely the modified Hestrin-Schramm culture medium in presence of 0.5% Ascorbic acid (Keshk, 2014b). The culture was incubated statically at 28°C in the liquid medium at pH 6 for one week.

2.2. Pellicles Production and Purification

BC membrane was produced by different *Gluconacetobacter xylinus* strains in 30 ml of HS and HSA media at 28°C for 7 days using 90-mm (i.d.) petridishes. After incubation, the pellicles produced on the surface of each medium were harvested and washed with water, hot 2% sodium dodecyl sulfate (SDS) and water successively, then dried on a Teflon plate.

2.3. Extraction of Cellulose from Cotton Stalks

Cotton stalks were ground by hammer mill. The ground CS was refluxed with 6% sodium hydroxide for two hours. The produced pulp was bleached with hypochlorite (3%) in two stages for 1.5 hrs at 60°C then filter and washed till neutralization.

2.4. Mercerization of Cellulose

Cellulose samples were soaked in 20% NaOH for 48 hrs and then washed with deionized water well till neutralization.

2.5. Preparation of Cellulose Phosphonic Dichloride

Mungall et al (1974) preparation procedure was modified as follow: 1.0g of dry mercerized BC or CS samples were suspended in cold dry pyridine (5°C) for 12 hrs, and then

add dropwise mix of 5 ml of phosphorus oxy-chloride in 25ml methylene chloride. The contents were heated for 2 hrs. Then, the contents were cooled and poured onto ice and filtered. The filtered was washed with 0.1N HCl, and deionized water till neutralization.

2.6. Determination of Phosphorus Content

Cellulose phosphonic dichloride (0.2g) was boiled in conc. HNO_3 till dissolution and then cooled and diluted to 100 ml with deionized water. Phosphorus was determined in the solution using ICP-AES Yvon J4185 spectrophotometer.

2.7. Determination of Metal Ion Uptake

BC, CS and their phosphonic dichloride derivatives (0.2g) were stirred in metal ion (Cr^{+3} , Fe^{+3} , Cu^{+2} , Zn^{+2} and Pb^{+2} ; 20 μg of each) solutions (25ml), for 30 min. The mixture was filtered and the remaining metal ions in the filtrate were determined. Metal ion uptake and phosphorus was determined in the solution by using ICP-AES technique (Inductive coupled plasma-Atomic Emission spectrometry).

2.8. FT-IR Spectroscopy Measurement

IR spectra were measured using a Nihon FT-IR-480 spectrometer. The reproducibility of the spectra was verified on three-sample preparation; from 64 to 100 scans were taken with a resolution of

2.9. Mass Spectral Measurement

Mass spectrum of cellulose phosphonic dichloride was recorded on Finningan SSQ 7000 spectrometer.

2.10. X-ray Diffractometry

The thick sample was prepared according to Kai & Keshk (1998) Diffractogram of the sample was recorded at room temperature with RIGAKU PRINT 2200V series using Ni-filtered CuK_α radiation ($\lambda = 1.54 \text{ \AA}$). The operating voltage and current were 40 Kv and 30 mA, respectively. Crystallinity index (C.I.) was calculated from the reflected intensity data using the Segal et al, method, according to Eq. (1)

$$\text{C.I.} = (I_{020} - I_{\text{am}}) / I_{020} \quad (1)$$

Where I_{020} and I_{am} represent the maximum intensity of the lattice diffraction and the intensity at $2\theta=18^\circ$, respectively.

3. Results & Discussion

Inferred spectra of BC and CS were recorded in the region of 4000 to 500 Cm^{-1} . The relative absorbance of the subscript wave number to the absorbance of the wave number at 1328 Cm^{-1} which is corresponding to the CH rocking of the ring was calculated for samples (El-Sayed et al, 1997). The asymmetry index (It was defined as the ratio of the band width at 4000-3000 Cm^{-1} region) on the low and high frequency side of the maximum at half band absorbance the bonded hydroxyl stretching gives

unsymmetrical band with broadening towards the lower frequency (Nada et al, 1990, 2000). On the other hand, the mean strength of the H-bonds (MBHS) was calculated as the ratio of A_{OH}/A_{CH} , where A. is the absorbance of the stretching vibration of subscript groups. Whereas the C.I. was calculated (the ratio of the absorbance of band at 1430 Cm^{-1} to band at 900 Cm^{-1}) (Nada et al, 2000). Table (1) shows the maximum absorption band of stretching vibration of OH groups (A_{OH}), MHBS, asymmetry index and C.I. of BC and CS. From Table (1), the asymmetry index reveals that the hydroxyl groups of the two kinds of cellulose are not free but entering into different modes of hydrogen bonds. Also, MHBS in case of BC is stronger than that in CS. This was confirmed by increasing in C.I. of BC rather than that in CS (Table 1). The relative absorbance of ether linkage (at 1120 Cm^{-1}) in BC has the lower value than that of CS. This means that, BC has the lower degree of polymerization (DP) than that of CS. On the other hand, CS has the highest relative absorbance, revealing its high DP. The relative absorbance of primary OH at 1035 Cm^{-1} of CS has a higher value than that of other kind of cellulose; it can be attributed to the higher DP than those of other two kinds (Table 1). These results are confirmed by measuring the DP as listed in Table (1). Figure (1) showed the heavy metal

ions uptake by BC and CS. It is clear that, the metal ion uptake by BC was higher than that of CS. This may be attributed, to the highest porosity, hydrophilicity and the largest surface area of BC (Keshk, 2011). These phenomena of BC increase intrinsic absorption and columbic interaction of BC with heavy metals. The incorporation of phosphonic dichloride group onto BC or CS increases their efficiencies toward metal ions uptake (Figures 2, 3). This may be attributed to, the presence of phosphonic dichloride that facilitates chemical reaction of heavy metals with cellulose. BC has the highest phosphate content, where, BC has the lowest degree of polymerization that increases the reactive end groups of cellulose. The highest phosphate content in BC shows the lowest in relative absorbance of ether linkage in FT-IR spectrum, which means more degradation in ether linkage. The presence of pyridine in phosphonation process is important owing to its reaction with HCl that produced during phosphonation process. FT-IR of the incorporated phosphate groups onto BC recorded two new bands at wave lengths of 1198 and 960 Cm^{-1} those are related to C-O-P vibration band. Moreover, the quantity of incorporated phosphonic dichloride in BC is nearly related to the relative absorbance of these groups.

Table 1. Infrared Spectroscopy Properties of BC, CS and their phosphonic dichloride

Sample	$\nu_{\text{max.}}$ (OH)	MBHS	Asymmetric index	$\nu_{\text{max.}}$ (CH ₂)	$A_{\text{C-O-C}} / A_{\text{CH}_2}$	$A_{\text{OH}} / A_{\text{CH}_2}$	DP	C.I
BC	3883	6.8	0.98	1317	1.15	1.15	400	9
CS	3275	2.4	0.78	1319	1.62	1.67	915	2.6
Phosphonic BC	3440	3.9	0.88	1297	1.00	0.98	320	7.8
Phosphonic CS	3200	1.9	0.66	1290	1.30	1.20	625	1.1

DP = Degree of polymerization

C.I = Crystallinity index

MBHS = mean hydrogen bonding

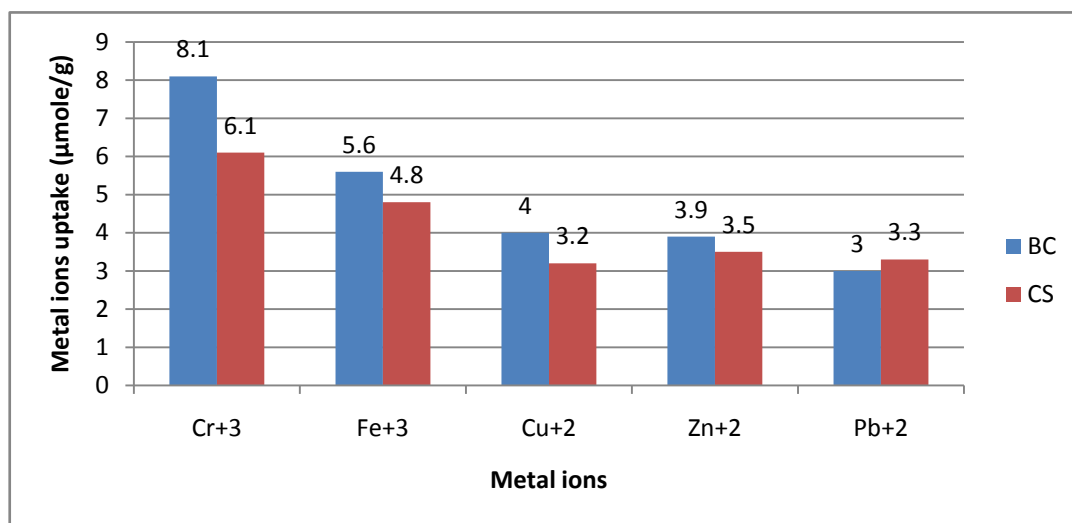


Figure 1. Metal ions uptake (µmole/g) by Bacterial Cellulose (BC) and Cotton Stalks (CS) in 25 ml of metal ions solution

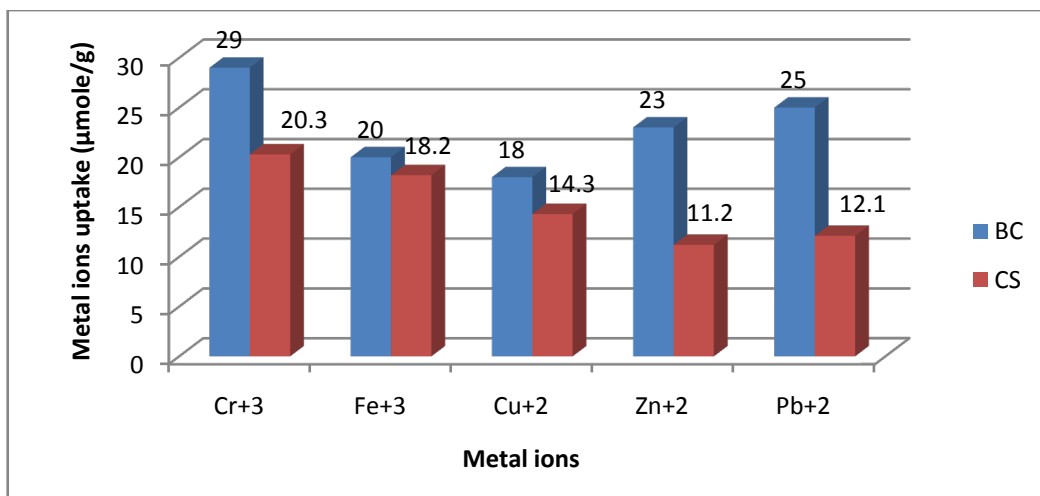


Figure 2. Metal ions uptake (µmole/g) by Bacterial Cellulose (BC) and Cotton Stalk (CS) in presence of Pyridine

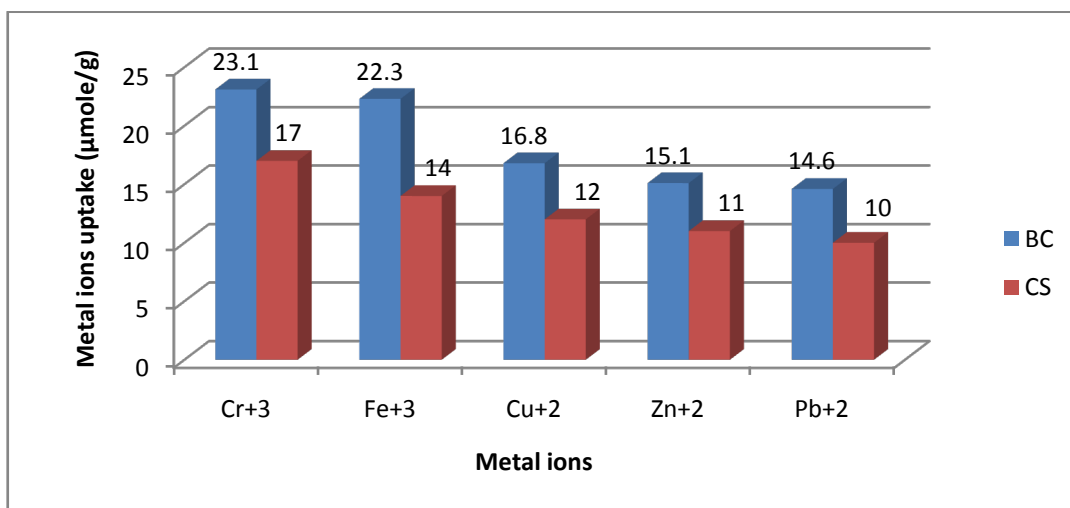


Figure 3. Metal ions uptake (µmole/g) by Cellulose 6-Phosphonic dichloride of Both Bacterial Cellulose (BC) and Cotton Stalks (CS)

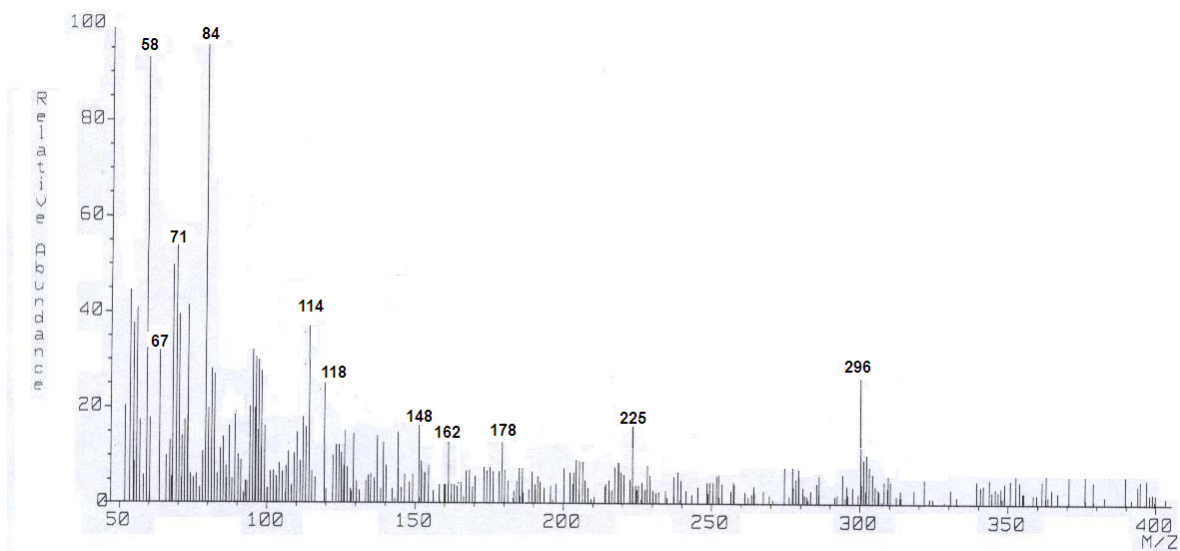
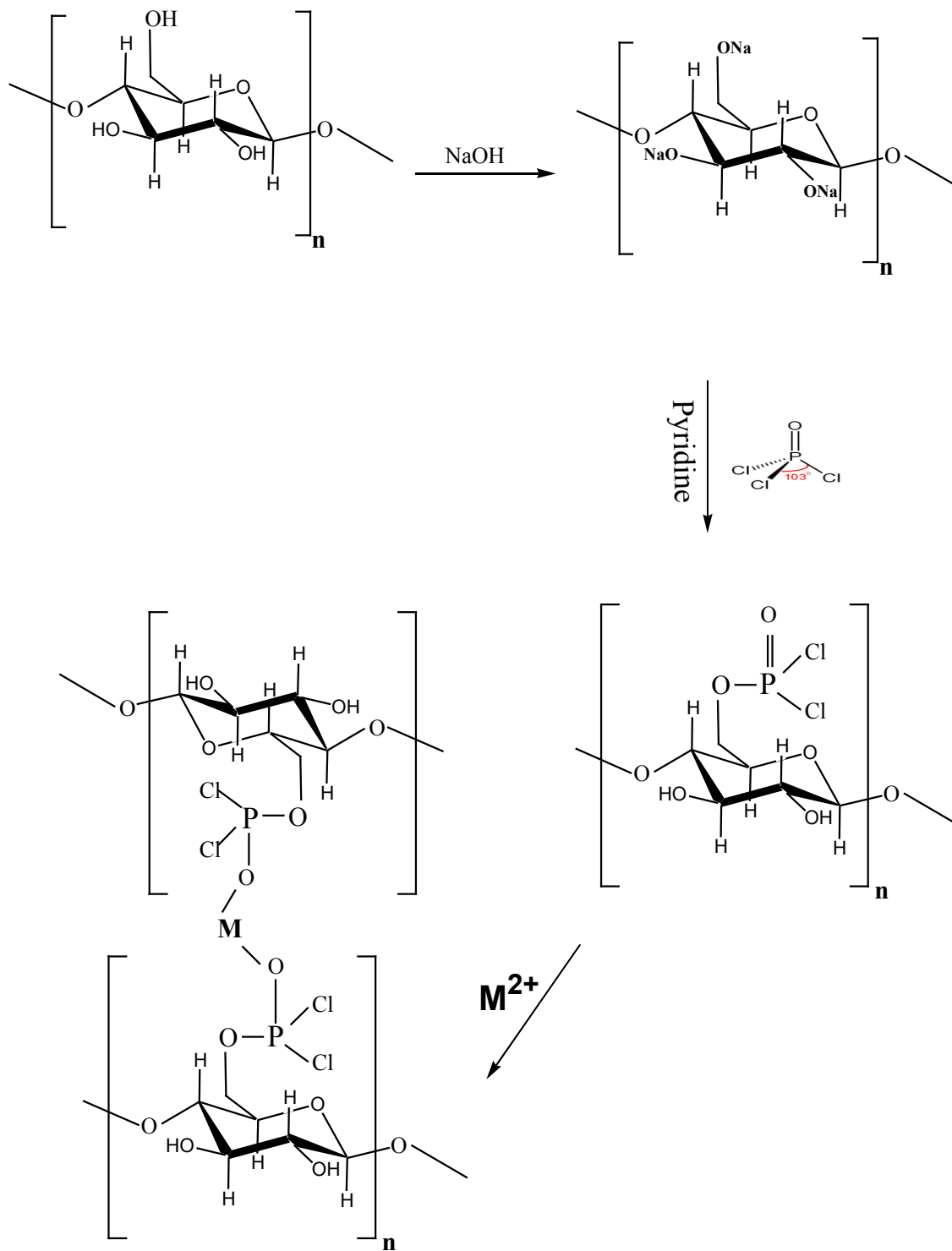


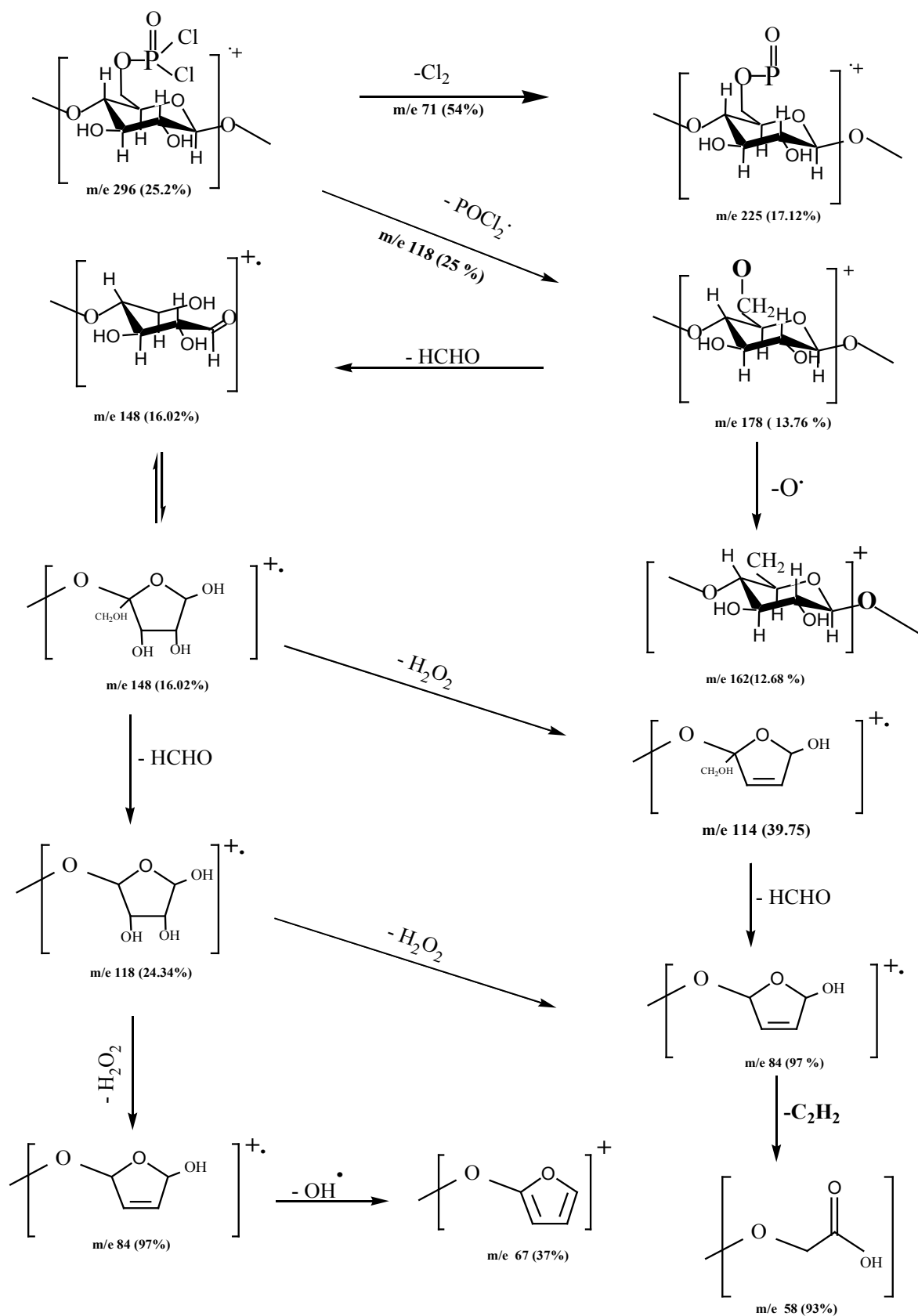
Figure 4. Mass Spectra of Cellulose Phosphonic Dichloride

The mass spectrum (Figure 4) revealed a radical cation peak at 296 (25.2%) corresponding to $[\text{C}_6\text{H}_{10}\text{O}_7\text{PCl}_2]^+$, a radical peak at 118 (25%) equivalent to $[\text{POCl}_2]$, and a major radical cation peak at 84(96%) corresponding to

$[\text{C}_3\text{H}_5\text{O}_3]^+$. The major fragmentation pattern of one unite of cellulose phosphate is represented in Scheme (2). These results prove the presence of mono substituted cellulose as cellulose 6- phosphonic dichloride.



Scheme 1. Reaction Mechanism of Cellulose 6-Phosphonic dichloride



Scheme 2. Fragmentation Pattern of the Cellulose 6- Phosphonic dichloride

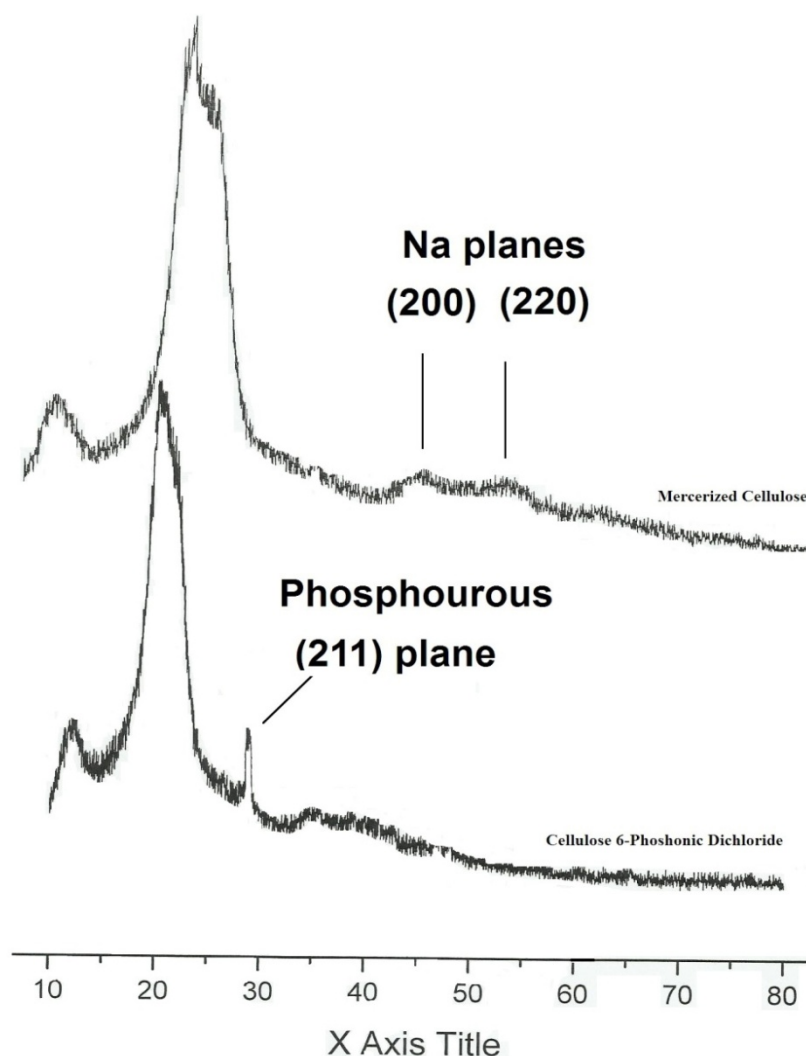


Figure 5. X-ray Diffractogram of both Mercerized Cellulose and Cellulose 6-Phosphonic Dichloride

Furthermore, the X-ray diffractograms of mercerized CS and its phosphonic dichloride samples are shown in Figure (5). The crystal kind change from cellulose I to cellulose II was observed, and the peaks shifted to lower Bragg angles, suggesting that the crystallite sizes decreased with alkali treatment. The calculated C.I. values of BC, CS, mercerized BC, mercerized CS and their phosphated dichloride samples were 88%, 67%, 80, 65 and 66%, respectively. These differences in the crystallinities are attributed to the different crystal lattices of the cellulose crystal structures. In mercerized cellulose pattern (Figure 5) sodium planes (200) and (220) are recorded at $2\theta = 46^\circ$ and 55° , respectively. Whereas, in cellulose 6-phosphonic dichloride, phosphorous plane (211) is observed at $2\theta = 30^\circ$ (Hu et al 2009). From these data, it is confirmed the presence of phosphorous as well as confirmed by mass spectrum.

4. Conclusions

BC differs from cotton stalks with respect to its high crystallinity and purity from lignin. BC is more reactive

towards phosphonation rather than that of CS owing to its high crystallinity (88%) with low degree of polymerization (890). Furthermore, BC has the highest ion exchange efficiency. Incorporation of phosphate group onto BC and CS increases their efficiency towards metal ion uptake.

REFERENCES

- [1] Ahmed, S., Chughtai, S., & Keane, M. A. (1998). The removal of cadmium and lead from aqueous solution by ion exchange with Na-Y zeolite, *Separation. Purification. Technology.* 13, 57–69.
- [2] Brown, Jr. R. M., Willison, J. H. M., & Richardson, C. L. (1976). Cellulose biosynthesis in *Acetobacter xylinum*. *Proceeding. Nature. academic. Science. USA*, 73, 4565-4569.
- [3] Colvin, J.R. (1980). The biosynthesis of cellulose: In "Plant biochemistry", (Priess, J. ed.), p 543-570. Academic Press, Inc., New York, USA.

- [4] Delmer, P. D., & Yehudit, A. (1995). Cellulose biosynthesis. *The Plant Cell*, 7, 987-1000.
- [5] El-Saied H, Basta A, Abdel-nour K (1997). Some properties of wood pulp, polymer complexes paper sheet. *Int. J. Polym. Mat.* 36. 131-149.
- [6] Erdem, E., Karapinar, N., & Donat, R. (2004). The removal of heavy metal cations by natural zeolites. *Journal of Colloid and Interface Science*, 280, 309–314.
- [7] Goswami, A., & Singh, A. K. (2002). Silica gel functionalized with resacetophenone: Synthesis of a new chelating matrix and its application as metal ion collector for their flame atomic absorption spectrometric determination. *Analytical. Chime Acta*, 454, 229–240.
- [8] Kaşgöz, H., Durmuş, A., & Kaşgöz, A. (2008). Enhanced swelling and adsorption properties of AAm-AMPSNa/clay hydrogel nano-composites for heavy metal ion removal. *Polymers for Advanced Technologies*, 19, 213–220.
- [9] Kai, A. & Keshk, S.M.A.S. (1998). Structure of Nascent Microbial Cellulose I, *Polymer Journal*, 30, 996.
- [10] Kai, A. & Keshk, S.M.A.S. (1999). Structure of Nascent Microbial Cellulose II, *Polymer Journal* 31, 61.
- [11] Keshk, S. M. A. S., & Nada, A. (2003). Heterogeneous Derivatization of Bacterial and Plant Celluloses. *Biotechnology. Bioscience. Asia*, 1, 39.
- [12] Keshk, S. M. A. S., & Nada, A. (2008). Chemical modification of some cellulosic wastes for increasing their efficiencies as ion exchangers. *Egypt. J. Chemistry*. 50 (3).
- [13] Keshk, S. M. A. S. (2008). Homogenous Reaction of Cellulose from Different Natural Sources. *Carbohydrate Polymer*. 74, 942-945.
- [14] Keshk, S.M.A.S. (2011). Scope on Bacterial Cellulose Production. LAMBERT Academic publishing. ISBN 978-3-8443-0211-0.
- [15] Keshk, S.M.A.S & Haijia, M (2011) A New Method for Producing Microcrystalline Cellulose from *Gluconacetobacter xylinus* and Kenaf. *Carbohydrate Polymer*, 84, 1301-1305.
- [16] Keshk, S. M. A. S. (2014a). Vitamin C Enhances Bacterial Cellulose Production in *Gluconacetobacter Xylinus*. *Carbohydrate Polymer*, 99, 98-100.
- [17] Keshk, S.M.A.S (2014b) Bacterial Cellulose Production and Its Industrial.
- [18] Applications. *Bioprocessing & Bio techniques* 4,15, 2014.
- [19] Klemm, D., Philipp, B., Heinze, T., Heinze, U., Wagenknecht, W. (1998). *Comprehensive Cellulose Chemistry, Volume 1, Fundamentals and Analytical Methods*, Wiley-VCH, Weinheim.
- [20] Hu, Y., Yusufoglu, Y., Kanapathipillai, M., Yang, H., Wu, Y., Thiagarajan, P., Deming, T., Akinc, M. Schmidt-Rohrb, K. and Mallapragada, S. (2009). Self-assembled calcium phosphate Nano composites using block polypeptide templates. *Soft Matter*, 5, 4311–4320.
- [21] Mercier, L., & Detellier, C. (1995). Preparation, characterization and applications as heavy-metals sorbents of covalently grafted thiol functionalities on the interlamellar surface of montmorillonite. *Environ. Sci. Technol.* 29, 1318–1323.
- [22] Mercier, L., & Pinnavaia, T. J. (1998). A functionalized porous clay hetero structure for heavy metal ion (Hg^{2+}) trapping microporous and mesoporous materials, *Micropor. Mesopor. Mater.* 20, 101–106.
- [23] Mungall, W.S., Greene, G.L., Miller, P.S. and Letsinger, R.L (1974). Use of phosphorus oxychloride in synthesizing nucleotides and oligonucleotides. *Nucleic Acids Res.* 1, 615–627.
- [24] Nada, A., Shabaka, A., Yousef, A., & Nour, A. (1990). Infrared spectroscopic and dielectric studies of Swollen cellulose. *Journal of Applied Polymer Science*, 40, 731-739.
- [25] O'Connell, D. W., Birkinshaw, C., & O'Dwyer F. T. (2008). Heavy metal adsorbents prepared from the modification of cellulose: A review. *Bioresource Technology*, 99, 6709–6724.
- [26] Okieimen, F. E., Sogbaiké, C. E., & Ebhoaye, J. E. (2005). Removal of cadmium and copper ions from aqueous solution with cellulose graft copolymers. *Separation and Purification Technology*, 44, 85–89.
- [27] Padilha, P. M., Gomes, L. A. M., C. C., Padilha, F., Moreira, J. C., & Filho, N. L. D. (1999). Determination of metal ions in natural waters by flame-AAS after pre-concentration on a 5-amino-1,3,4-thiadiazole-2-thiol modified silica gel. *Analytical. Letter*. 32, 1807–1820.
- [28] Rivas, B. L., Maturana, H. A., Villegas, S., & Pereira, S. (1998). Highly Pb(II)-selective resin based on cross linked poly (acrylamido glycolic acid) copolymer, *Polymer Bulletin*., 40, 721–728.
- [29] Zhou, D., Zhang, L., Zhou, J., & Guo, S., (2004). Cellulose/chitin beads for adsorption of heavy metals in aqueous solution. *Water Research*. 38 (11), 2643–2650.