International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 3S, 2025
https://www.theaspd.com/ijes.php

Development And Comprehensive Characterization Of Cnc-Pectin Biocomposite With Enhanced Antimicrobial Activity

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ABSTRACT
Cellulose nanocrystals (CNCs) were added to a pectin matrix to improve its structural, thermal, and antibacterial qualities, creating a unique bio composite film. The bio composite was characterized using various analytical techniques. Fourier Transform Infrared Spectroscopy (FTIR) confirmed successful interactions between CNCs and pectin through hydrogen bonding. Scanning Electron Microscopy (SEM) revealed a uniform dispersion of CNCs within the pectin matrix, improving the composite's surface morphology. EDAX confirmed the presence of key elements such as carbon (C), oxygen (O). X-Ray Diffraction (XRD) analysis demonstrated an increase in crystallinity with CNC incorporation, contributing to enhanced mechanical properties. Furthermore, antimicrobial studies indicated significant inhibitory effects against Gram-positive and Gram-negative bacteria, suggesting potential applications in food packaging and biomedical fields. The results highlight the promising role of CNC-doped pectin bio composites as sustainable materials with improved physicochemical and functional properties.
Key words : bio composite, antimicrobial, biomedical, bacteria , cellulose nanocrystals

1.INTRODUCTION

Nanotechnology has rapidly developed and serves as an exceptional tool for various biomedical applications, including tissue regeneration, biosensors, drug delivery, and antimicrobial uses [1] Nano-materials offer unique physicochemical properties due to their larger surface-to-volume ratio and a higher number of atoms present near the surface. These advantages can be leveraged to design specific materials capable of interacting with tissues to achieve maximum antimicrobial efficacy with minimal toxicity possible [2]. The world is concerned about the serious environmental issues, such as the growing challenges of disposing of garbage and the growing threat of global warming brought on by the non-biodegradability of certain polymers used in packaging and agriculture. In order to address the issues brought about by plastic trash, numerous attempts have been made to find an environmentally benign material. Environmental concerns have made the development of new biodegradable polymers a significant emerging area of research in both business and academia. [3-5]

The most prevalent natural biopolymer is cellulose, which may be easily obtained from renewable resources. Fibrous cellulose is used to make hemp rope, cotton, and wood. Repeated units of the monomer glucose make up cellulose. Given the extraordinary demand for environmentally friendly and biocompatible products, cellulose is thought to be a nearly limitless raw material with intriguing forms and qualities. The goal of chemically functionalizing cellulose is to modify its characteristics for various uses, especially as a chemical feedstock for the synthesis of cellulose derivatives for a range of applications etc [6-7]. Sono chemical degradation and enzymatic degradation are appealing methods for recycling or degrading cellulose-based goods. The production of CNCs has made use of a variety of cellulose-rich raw materials [8]. Several methods have been employed in the past to prepare CNCs. The cellulose nanoparticles' size, shape, morphology, and structural characteristics are established using these techniques. One popular technique for separating CNCs from amorphous cellulose is acid hydrolysis.

However, because of the leftover acid, using acid can potentially degrade cellulose and make it more prone to corrosion [9-10]. Emulsions, biomedical devices, packaging, and rheology modifiers such as hydrogels for biomedicine, bio composite films, supercapacitors, and xero-gels are all made with nanocellulose [11-17].

A diverse collection of polysaccharides, pectin has a number of substructures, including neutral sugar side chains, rhamnogalacturans I and II, partly methyl-esterified homogalacturan, and xylogalacturan. [18] This polysaccharide controls the porosity of the cell wall, serves as the primary adhesive between neighbouring cells, and creates a matrix for the deposition and growth of the cellulosic-glycan network in plant cell walls. [19] There are numerous uses for pectin in the Savory and pharmacological sectors. [20-21] For example, it functions as a gelling medium, thickening, texturizer, emulsifier, stabilizer, and sugar substitute in diet beverage formulations. It is also used in the production of a number of food products, such as jams, jellies, candies, syrups, preserves, sauces, etc. In the chemical industry, pectin is also used as a drug carrier in film-coated tablets, gel beads, and matrix [22].

To replicate the structure of plant cell walls, pectin and cellulose composites have been created [23-24] Pectin-cellulose mixtures are currently used in water purification and food wrapping due to their versatility [25-27]. Low levels of CNC have been used with pectin to create less water-permeable films that are edible [28]. Additionally, both polysaccharides have been utilized as hydrogels to study the mineralization of hydroxyapatite and as excipients with alginate to make probiotic tablets [29-30].

The Greek words "Borassus" and "flabellifer," which translate to "fruit with leather covering" and "fan-bearer," respectively, are the origins of the name Borassus flabellifer, often known as the palmyra palm. Southeast Asia and the Indian subcontinent are the known origins of Borassus flabellifer. Additionally, it is regarded as a permanent gift from nature that can thrive in arid and semi-arid environments and endure any unfavourable weather conditions [31].

In order to extract CNCs, mesocarp fibre from Borassus flabellifer was subjected to acid hydrolysis in the presence of ultrasound sonication. The extracted CNCs and pectin were blended together to form a bio composite.

2. EXPERIMENTAL PROCEDURE

2.1 Materials

Mesocarp fibre from Borassus flabellifer was gathered from a local market in India. Sigma-Aldrich supplied concentrated sulphuric acid (H_2SO4, 98%). Sodium hydroxide pellets, sodium hypochlorite solution (NaOCl and pectin were obtained from Shiv Chemical Laboratory in India. Deionised water and ethanol were used.

2.2 PREPARATION OF CELLULOSE NANOCRYSTALS

Borassus flabellifer's mesocarp fibre was processed into powder. 2g of powdered mesocarp fibre was washed with distilled water to eliminate contaminants. The dried sample was bleached with 25% NaOCl and heated for approximately three hours. The bleaching process was carried out to purify the material. The resulting cellulose was rinsed with deionised water and dried at room temperature.

The dried sample was treated with concentrated H_2SO_4. 25ml of 98% concentrated H_2SO_4 was added to the dried sample. It was left undisturbed for three hours. To terminate the reaction, the mixture was placed in a cold ice bath. The pH was raised to 7 by adding an excess of NaOH and sonicating for approximately 120 minutes. The sample was rinsed with ethanol and deionised water, filtered, and dried in a hot air oven at 80º C for 36 hours.

2.3 PREPARATION OF CNC-PECTIN BIOCOMPOSITE

1.8g of pectin in 25ml water was stirred for 30 minutes at room temperature. 0.2g of CNC was stirred for 15 minutes and later sonicated for 30 minutes. Both the stock solutions were mixed and stirred for 20 minutes at room temperature to form a bio composite film. The resulting solutions were cast in to a polystyrene petri dish and allowed to dry for 48 hours at 50ºC. A thin bio composite film was formed.

3. RESULTS AND DISCUSSION

3.1 FOURIER TRANSFORM INFRARED SPECTRAL ANALYSIS

The molecular interactions and chemical bonding between pectin and CNC in bio composite materials are commonly investigated using Fourier-transform infrared (FT-IR) spectroscopy. FTIR spectrum of CNC-Pectin bio composite is shown in fig 1. FT-IR spectrum of a CNC-pectin bio composite provide clarity on the molecular interactions between these two materials and assist in determining the functional groups at effect. When CNC and pectin were combined to form a bio composite, shifts in the peak positions or changes in intensity can occur, which suggest interactions between the hydroxyl groups of cellulose nanocrystals and the carboxyl groups of pectin. These interactions, such as hydrogen bonding or electrostatic interactions, contribute to the overall stability and mechanical properties of the bio composite.

The broad peak around 3414.67 cm^{-1} suggests the presence of O-H stretching, indicative of alcohols. Peaks at 2922.50 cm^{-1} and 2852.41 cm^{-1} are associated with C-H stretching vibrations, commonly seen in alkanes or aliphatic compounds. The peak at 1637.42 cm^{-1} could be attributed to C=C stretching, indicating the presence of alkenes or aromatic rings. Peaks at 1560.40 cm^{-1} and 1441.12 cm^{-1} might correspond to aromatic ring vibrations or bending modes of C-H bonds. The region between 1311.24 cm^{-1} and 1020.03 cm^{-1} includes peaks that are often associated with C-O stretching or C-H bending vibrations, pointing to esters, ethers, or alcohols. Peaks below 1000 cm^{-1}, such as at 874.93 cm^{-1} and 669.65 cm^{-1}, can represent bending vibrations or out-of-plane deformations in aromatic compounds or other structural features.

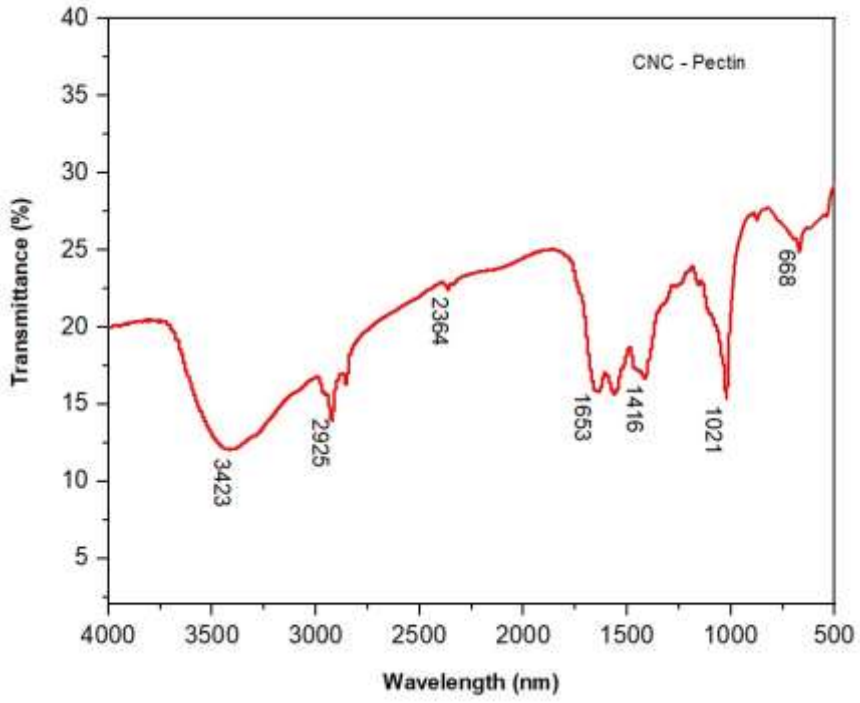

Fig 1: FTIR spectrum of CNC-Pectin Bio-Composite

International Journal of Environmental Sciences
ISSN: 2229-7359
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3.2 SCANNING ELECTRON SPECTROSCOPY

Scanning Electron Microscope (SEM) image which is shown in fig 2 depicts a surface with numerous elongated and needle-like objects distributed randomly over the field of view. These structures appear crystalline, indicating the creation of a crystalline phase, most likely through a chemical or physical deposition process. The placement and spacing of the crystals appear arbitrary, with smaller granular characteristics scattered around the surface, possibly reflecting spots of nucleation or impurities. This microstructure could be a salt, a layer of precipitate, or another crystallised chemical, which is common in applications such as thin-film coatings, corrosion investigations, and material fabrication.

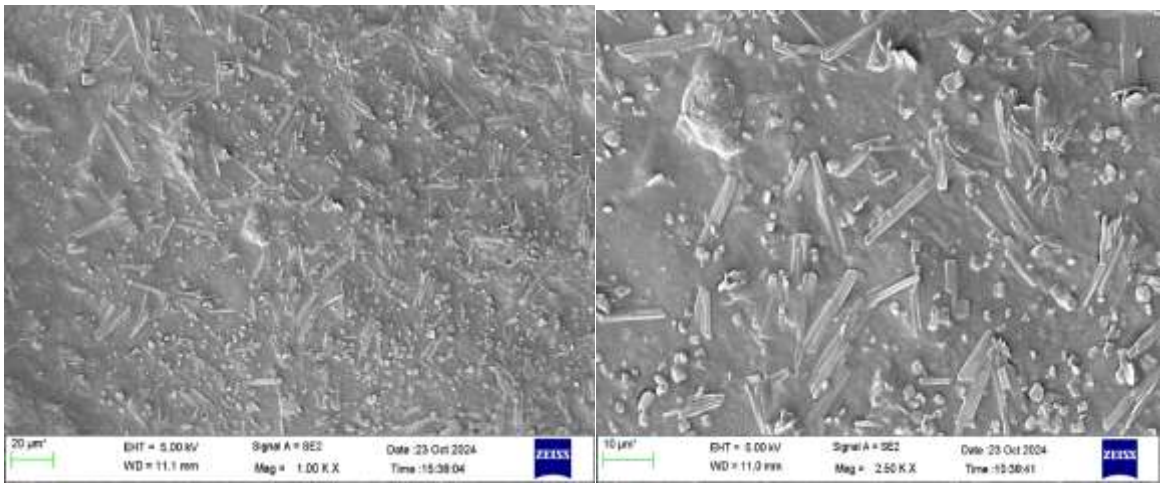

Fig 2: SEM images of CNC-Pectin Bio-Composite

3.3 ENERGY DISPERSIVE X-RAY ANALYSIS (EDAX)

The EDAX spectrum of CNC-Pectin bio- composite is shown in fig 3. The elemental makeup of a sample can be seen in this Energy Dispersive X-ray Spectroscopy (EDS or EDAX) spectrum. The intensity of detected X-rays is shown on the y-axis, while energy (in keV) is represented on the x-axis. The spectrum displays strong peaks at low energy values, with labels indicating elements detected in the sample. The significant peaks for Carbon (C) and Oxygen (O) show that the material examined is rich in these elements, presumably indicating an organic or oxide-based makeup. There may not be many heavier elements present because there are no notable peaks at higher energy levels. Table 1 shows the weight and atomic percent of the elements present in bio composite.

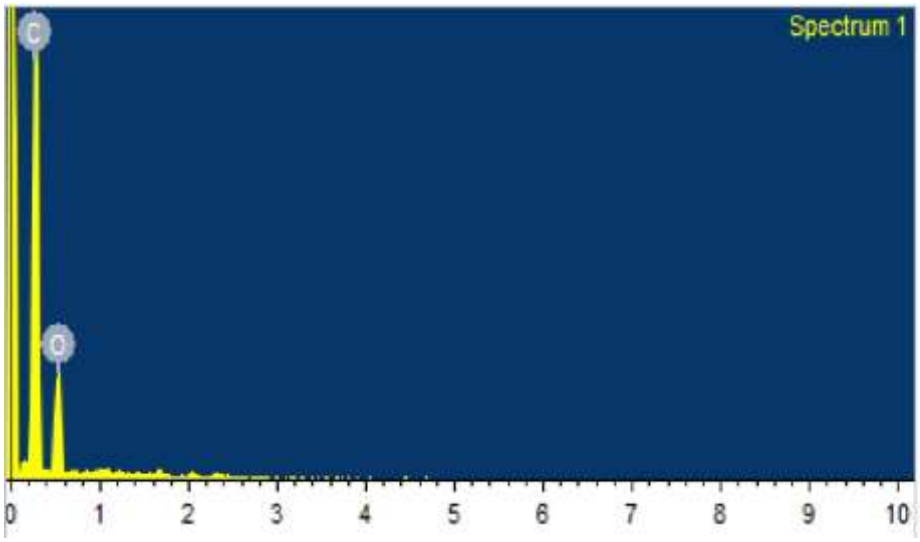

Fig 3: EDAX of CNC-Pectin Bio-Composite

Element	Weight %	Atomic %
C	65.8	76.3
O	34.2	23.7

Table 1: EDAX of CNC-Pectin Bio-Composite

3.4 X-RAY DIFFRACTION METHOD

X-ray diffraction pattern, which is used to determine a material's crystalline structure is shown in fig 4. The main peaks were detected at 2θ = 9.464° and 18.851°. The peaks correlate to the material's crystalline planes. The broad nature of the peaks indicates that they are composed of nanocrystalline. The Scherrer Equation is applied to estimate particle size based on XRD peak broadening.

$$D=K\lambda/\beta\cos\theta$$

where, D is the crystalline size, K is Braggs constant, β is Full width at half maximum, θ is Bragg angle, and λ is X-ray wavelength.

The Scherrer Equation yields an estimated particle size of around 10.07 nm. This shows that the material is composed of nanocrystals, as indicated by the large peaks in the pattern produced by XRD. The Crystallinity Index (CI) was used to calculate the degree of crystallinity in the substance. Segal's approach is a standard way to calculate it based on an XRD pattern.

$$CI = \frac{(I_c - I_{am})}{I_c} \times 100$$

I*crystalline* is the intensity of the main crystalline peak (at 2θ = 18.851°).

I*amorphous* is the minimum intensity between the two major peaks, representing the amorphous phase. The estimated Crystallinity Index for the given XRD graph is 80%. This indicates that the sample has a high degree of crystallinity, with about 20% amorphous content.

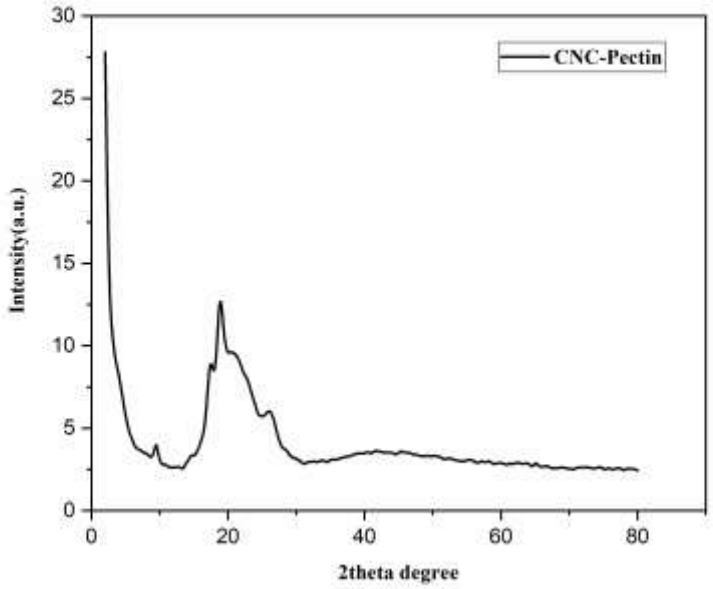

Fig 4: XRD graph of CNC-Pectin

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3.5 ANTI-BACTERIAL STUDY OF CNC-PECTIN BIO-COMPOSITE

Kirby-Bauer test is widely used to determine the sensitivity or resistance of bacteria and fungi to various antimicrobial compounds, and it uses the Muller Hinton agar. Muller-Hinton agar is a non-selective, non-differential medium capable of growing a wide range of non-fastidious organisms. Kirby-Bauer test is also known as disk diffusion method.

The acquired CNC-Pectin BioComposite are given to antibacterial research of two positive and two negative bacteria, as indicated in Fig 5, and the results are analysed. A larger value in the sample compared to the control shows that the bacteria are sensitive to the synthesized bio composite, whereas a lower value indicates resistance. In this scenario, the bio composite's values are greater than the control's. As a result, the bacteria are sensitive to the prepared bio composites. This reveals that bio composites have high antibacterial characteristics, making them ideal for extensive use in the medical business. If the prepared bio composite's properties are further studied and improved, it may be used in place of strong antiseptics. As a result, the prepared CNC-Pectin bio composite shows good resistance to these bacteria. Table 2 shows the activity of CNC-Pectin bio composite.

Gram negative Bacteria :

Gram positive Bacteria :

Fig 5: Anti-Bacterial activity of CNC-Pectin Bio-Composite

Bacteria	CNC-PECTIN Bio-Composite	Control (amikacin)
Escherichia coli	16mm	16mm
Proteus vulgaris	14.5mm	17mm
Bacillus subtilis	15mm	14mm
Staph aureus	16.2mm	15mm

Table 2: Activity of positive and negative bacteria

3.6 ANTIFUNGAL ACTIVITIES OF CNC-PECTIN BIO-COMPOSITE

The antifungal activity of CNC-Pectin are investigated against Candida albicans and Aspergillus niger. Bio composite showed minimum activity against these fungi which was shown in fig 6. Table 3 shows the activity of CNC-Pectin bio composite.

Fig 6: Anti-fungal activity of CNC-Pectin Bio-Composite

Fungus	CNC-PECTIN Bio-Composite	Control (Nystatin)
Candida albicans	13.6mm	14mm
Aspergillus niger	8mm	13mm

Table 3: Activity of fungi

4.CONCLUSION

The structural, elemental, and functional characteristics of CNC-Pectin bio composite are elucidated by means of characterization and analysis using a variety of techniques, including FTIR, SEM, EDAX, XRD, and antimicrobial tests. FTIR Analysis confirmed the successful interaction between CNC and Pectin, showing characteristic peaks corresponding to hydroxyl, carboxyl, and ether functional groups, indicating strong intermolecular bonding. SEM Analysis revealed a well-distributed and homogeneous surface morphology, demonstrating good CNC dispersion within the Pectin matrix, with minimal agglomeration. The elemental composition was confirmed by EDAX Analysis, which also ensured the

purity of the bio composite by proving the presence of vital components like carbon and oxygen. The bio composite was found to be semi-crystalline by XRD analysis, with a rise in crystallinity brought about by CNC inclusion, which improves mechanical and thermal stability. Significant antibacterial activity was shown in antimicrobial tests, indicating that CNC-Pectin bio composite may be a potential material for use in food preservation, packaging, and biomedical applications. This study demonstrates that CNC-Pectin bio composites are appropriate for a range of industrial and biological applications due to its superior structural integrity, enhanced crystallinity, and potent antibacterial qualities.

5.ACKNOWLEDGEMENT

I extend my sincere thanks to Karunya Institute of Technology and Sciences, Coimbatore and Schudder Pvt.Ltd, Nagercoil.

6. CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this

article.

7. REFERENCES

[1] S. Aggarwal, "Nanotechnology in endodontics: current andpotential clinical applications," Endodontology, vol. 28, no. 1,p. 78, 2016.
[2] A. Shrestha and A. Kishen, "Antibacterial nanoparticles inendodontics: a review," Journal of Endodontics, vol. 42, no. 10,pp. 1417–1426, 2016.
[3] Taghizadeh et al. SpringerPlus, 2013,2,376.
[4] Bella G. R., Jeba Jeevitha R. and Avila Thanga Booshan Thermal and morphological characteristics of cellulose nanocrystals filled PVA/starch nanocomposites Journal of Chemical and Pharmaceutical Research, 2015, 7(8):928-934.
[5] J. Wróblewska-Krepsztul et al.Recent progress in biodegradable polymers and nanocompositebased packaging materials for sustainable environment Int. J. Polym. Anal. Charact. (2018)
[6] Varshney V.K, Sanjay Naithani,(2011), Cellulose Fibers, Bio and Nano Polymer composites, Springer-Veralag Berlin Heidelberg.
[7] E. Abraham, B. Deepa, L.A. Pothan, M. John, S.S. Narine, S. Thomas, R. Anandjiwala, Physicomechanical properties of nanocomposites based on cellulose nanofibre and natural rubber latex, Cellulose 20 (2013) 417–427.
[8] D. Gaspar, S.N. Fernandes, A.G. De Oliveira, J.G. Fernandes, P. Grey, R.V. Pontes, L. Pereira, R. Martins, M.H. Godinho, E. Fortunato, Nanocrystalline cellulose applied simultaneously as the gate dielectric and the substrate in flexible field effect transistors, Nanotechnology 25 (2014) 094008.
[9] J. Guo, X. Guo, S. Wang, Y. Yin, Effects of ultrasonic treatment during acid hydrolysis on the yield, particle size and structure of cellulose nanocrystals, Carbohydr. Polym. 135 (2016) 248–255.
[10] Y. Zhang, X.B. Lu, C. Gao, W.J. Lv, J.M. Yao, Preparation and characterization of nano crystalline cellulose from bamboo fibers by controlled cellulase hydrolysis, J Fiber Bioeng Inform 5 (2012) 263–271.
[11] O. Faruk, M. Sain, R. Farnood, Y. Pan and H. Xiao, J. Polym. Environ., 22, 279 (2014); https://doi.org/10.1007/s10924-013-0631-x
[12] L.O. Pinto, J.S. Bernardes and C.A. Rezende, Carbohydr. Polym., 218, 145 (2019); https://doi.org/10.1016/j.carbpol.2019.04.070
[13] E.S. Ferreira and C.A. Rezende, ACS Sustain. Chem. & Eng., 6, 14365 (2018); https://doi.org/10.1021/acssuschemeng.8b03071
[14] J.P.S. Morais, M.F. Rosa, M.M. de Souza Filho, L.D. Nascimento, D.M.do Nascimento and A.R. Cassales, Carbohydr. Polym., 91, 229 (2013); https://doi.org/10.1016/j.carbpol.2012.08.010
[15] Ú. Fillat, B. Wicklein, R. Martín-Sampedro, D. Ibarra, E. Ruiz-Hitzky, C. Valencia, A. Sarrión, E. Castro and M.E. Eugenio, Carbohydr. Polym., 179, 252 (2018); https://doi.org/10.1016/j.carbpol.2017.09.072

[16] Y. Liu, Y. Sui, C. Liu, C. Liu, M. Wu, B. Li and Y. Li, Carbohydr. Polym., 188, 27 (2018); https://doi.org/10.1016/j.carbpol.2018.01.093
[17] E. Espinosa, I. Bascón-Villegas, A. Rosal, F. Pérez-Rodríguez, G. Chinga- Carrasco and A.Rodríguez, Int. J. Biol. Macromol., 141, 197 (2019); https://doi.org/10.1016/j.ijbiomac.2019.08.262
[18] E. Chanliaud, M.J. Gidley In vitro synthesis and properties of pectin/Acetobacter xylinus cellulose composites Plant J. (1999), 10.1046/j.1365-313X.1999.00571.x
[19] W.G.T. Willats, L. Mccartney, W. Mackie, J.P. Knox Pectin: cell biology and prospects for functional analysis Plant Mol. Biol. (2001), 10.1023/A:1010662911148
[20] P. Srivastava, R. Malviya Sources of pectin, extraction and its applications in pharmaceutical industry - an overview Indian J. Nat. Prod. Resour., 2 (2011), pp. 10-18
[21] B.R. Thakur, R.K. Singh, A.K. Handa Chemistry and uses of pectin - a review Crit. Rev. Food Sci. Nutr. (1997), 10.1080/10408399709527767
[22] Ana González Moreno , Susana Guzman-Puyol , Eva Domínguez Pectin-cellulose nanocrystal biocomposites: Tuning of physical properties and biodegradability International Journal of Biological Macromolecules Volume 180, 1 June 2021 https://doi.org/10.1016/j.ijbiomac.2021.03.126
[23] E. Chanliaud, M.J. Gidley In vitro synthesis and properties of pectin/Acetobacter xylinus cellulose composites Plant J. (1999), 10.1046/j.1365-313X.1999.00571.x
[24] O.M. Astley, E. Chanliaud, A.M. Donald, M.J. Gidley Structure of Acetobacter cellulose composites in the hydrated state Int. J. Biol. Macromol. (2001), 10.1016/S0141-8130(01)00167-2
[25] E.F. Lessa, A.L. Medina, A.S. Ribeiro, A.R. Fajardo Removal of multi-metals from water using reusable pectin/cellulose microfibers composite beads Arab. J. Chem. (2017), 10.1016/j.arabjc.2017.07.011
[26] V. Bátori, M. Jabbari, D. Åkesson, P.R. Lennartsson, M.J. Taherzadeh, A. Zamani Production of pectin-cellulose biofilms: a new approach for citrus waste recycling Int. J. Polym. Sci. (2017), 10.1155/2017/9732329
[27] R.M. Viana, N.M.S.M. Sá, M.O. Barros, M. de F. Borges, H.M.C. Azeredo Nanofibrillated bacterial cellulose and pectin edible films added with fruit purees Carbohydr. Polym. (2018), 10.1016/j.carbpol.2018.05.017
[28] M. Chaichi, M. Hashemi, F. Badii, A. Mohammadi Preparation and characterization of a novel bionanocomposite edible film based on pectin and crystalline nanocellulose Carbohydr. Polym. (2017), 10.1016/j.carbpol.2016.09.062
[29] T. Huq, K.D. Vu, B. Riedl, J. Bouchard, J. Han, M. Lacroix Development of probiotic tablet using alginate, pectin, and cellulose nanocrystals as excipients Cellulose, 23 (2016), pp. 1967-1978, 10.1007/s10570-016-0905-2
[30]D.M. Catori, E.H. Fragal, I. Messias, F.P. Garcia, C.V. Nakamura, A.F. Rubira Development of composite hydrogel based on hydroxyapatite mineralization over pectin reinforced with cellulose nanocrystal Int. J. Biol. Macromol., 167 (2021), pp. 726-735, 10.1016/j.ijbiomac.2020.12.012
[31] Veilmuthu P. Palmyra – nature's perennial gift in the face of climate crisis; 2020. http://climatesouthasia.org/palmyra-natures-perennial-gift-in-the-face-of-climate-crisis/