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Water Hyacinth Cellulose/Silk Fibroin Composite Films: Preparation and Characterization

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This study aimed to extract cellulose from water hyacinth using as material for preparation of cellulose/silk fibroin composite films. Using scanning electron microscope (SEM), the native silk fibroin film has smooth surfaces and homogeneous texture, while the cellulose film has short fiber on its surface with dense in texture. The composite films have different morphologies depending on types of polymer used and hierarchical step of film. FTIR analysis showed that the main functional groups of both cellulose (hydroxyl groups) and silk fibroin (carbonyl and amine groups). The composite films also found the absorption peaks of the functional groups which confirmed the composite of both polymers. This results indicated that both polymers had well compatible *via* bonding formation between the functional groups. The composite films have the maximum temperature decomposition ($T_{d,max}$) slightly higher value than that of native silk fibroin film. This suggested that the interaction between silk fibroin and cellulose resulted to increase of thermal stability as well as the flexibility of the film.

Keywords: Water hyacinth, Cellulose, Silk fibroin, Composite film.

INTRODUCTION

In recent year, use-friendly and eco-friendly biopolymer based materials have been widely proposed, especially for agricultural and marine originated sources of raw materials for biopolymers [1-5]. Natural biopolymers, especially protein based materials have been used in wide range applications [6]. One of the most natural proteins used is silk fibroin derived from *Bombyx mori* [7-10]. It exhibits many promising characteristics for biomaterial applications both its properties and process cost [11-14]. The silk proteins could be regenerated into various forms depending on applications [15-18]. Among the silk fibroin forms, film has been widely used [19,20]. However, silk fibroin films were very brittle in the dry form and low mechanical strength [21]. This limitation could be improved by blending it with other polymers [21-24].

Cellulose is a homopolymer of glucose linked together *via* β-1,4-glycosidic bonds [25]. The structure of cellulose is a mixture of hemicellulose (20-30%) and lignin (15-30%) to form the complexation structure [26]. Recently, the cellulose is widely applied in various industries such as food [27], pharmacy [28], pulp and paper [29], water pollution treatment [30], or wine and beer [31]. The important source of cellulose is the cell wall of the plant [32].

In this work, water hyacinth was used as raw material for cellulose extraction. It is a fresh plant that rapidly grown and widely spread around the world [33]. The water hyacinth is a virulent cause of water pollution [34], however, it compose of a high content of cellulose [35]. The goal of this work is to extract cellulose from the water hyacinth for blending with the silk fibroin as composite films. The films were then characterized and discussed of their properties. The ability to generate such composite films would expand the range of properties and applications.

EXPERIMENTAL

Preparation of water hyacinth cellulose: The water hyacinth samples were collected from the pond situation in the Mahasarakham University campus then washed with tap water before cutting into small pieces. The samples were dried in an oven for 24 h and then crushed into powder. The cellulose was extracted according to previously reported [33] with some modifications. Water hyacinth powder (10 g) was digested by 100 mL 5% NaOH (w/v) with stirring and warming for 3 h. The mixture was then washed with distilled water until neutral. After dried at 90 ºC for 24 h, the dried sample was then bleached by 5% NaOCl (w/v) at room temperature for 24 h. The bleached samples were washed to neutral and dried again at 90 ºC for 24 h. Finally, the samples were hydrolyzed by 5% H_2SO_4 at 60 ºC for 8 h to obtain the cellulose solution. The cellulose solution was then stirred, washed with distilled water and filtered before use.

Preparation of silk fibroin solution: The cocoon of *B. mori* were firstly degummed with 0.5% (w/w) Na_2CO_3 solution at 100 ºC for 1 h to remove glue-like sericin and then washed with distilled water. The silk fibroin were then air-dried at room temperature before dissolving in a hot ternary solvent system of $CaCl_2$-ethanol-water (1:2:8 in mole ratio) for 5 h with stirring. The resulting silk fibroin solution was then dialyzed to remove solvent using cellulose tubular membrane (molecular weight cut off = 3-5 kDa) in distilled water for 3 days. The fresh distilled water was changed every day. The final silk fibroin concentrations after dialysis and filtration were adjusted to 1% (w/v) by distilled water.

Preparation of cellulose/silk fibroin composite films: The cellulose and silk fibroin solutions with different ratios (4:0, 3:1, 1:1, 1:3, and 0:4) were prepared and stirred homogeneously for 0.5 h. The mixture was then cast onto a 4.5 cm diameter petri dish followed by solvent evaporation at room temperature for 24 h. The films were peeled off and then placed in a desiccator until investigation.

Morphological analysis: All of the films were dehydrated and cut into ~1cm length before observing their morphology using a scanning electron microscope (SEM) (JEOL, JSM-6460LV, Tokyo, Japan). The film fractures were coated with gold (Au) to enhance conductivity before scanning.

FTIR analysis: The secondary structure of films was analyzed by Fourier transform infrared (FTIR) spectrophotometer (Perkin Elmer Spectrum Gx, USA) in the spectral region of 4000-400 cm^{-1} at 4 cm^{-1} spectral resolution and 32 scans with air as reference.

Thermal analysis: A thermogravimetric analyzer (TGA) (SDTQ600, TA-Instrument Co. Ltd., New Castle, USA) was used for thermal stability investigation of the films both native and composite films. In brief, 3-5 mg weighed films were heated from 50-800 ºC with 20 ºC/min of heating rate under nitrogen atmosphere.

Water solubility test: All the films (n = 3) were immersed into warm distilled water at 37 ºC for 24 h. After the water solubility treatment, the films were taken out and then dried in an oven at 100 ºC. The percentage of water solubility was an average of three measurements of each sample and calculated by dividing the weight loss by the initial weight.

RESULTS AND DISCUSSION

Transparency of films was observed from digital images as shown in Fig. 1. The native silk fibroin film (a) has smooth in surface and homogeneous throughout the film surface. The film has pale yellow, hard and thin as like as transparency than the native cellulose film (d). The cellulose (CE) film has pale white, smooth in surface, higher thickness and flexibility than the silk fibroin (SF) film. The SF/CE/SF (b) and CE/SF/CE (c) multilayers films have smooth surfaces, pale yellow/white followed by the colour of native polymer. The composite films have higher flexibility than the native silk fibroin film. This indicated that the cellulose reduced the brittle of silk fibroin.

SEM studies: Morphological observation of the films are shown in Fig. 2. At 1000X magnification, the silk fibroin film (a) has homogeneous and dense in texture with smooth surface. The SF/CE/SF composite film (b) appeared the cellulose fiber dispersed the film texture but the film still homogeneous in texture without phase separation. In case of the CE/SF/CE composite film (c), it found phase separation in the texture. However, the film still compacts together with the connection between the cellulose fibers. The native cellulose film (d) has less thickness more than other films with non-homogeneous in size and found non-woven of the fiber connecting between the texture of film.

FTIR studies: The secondary structure of films was investigated by FTIR spectrophotometer. This instrument specifically detected on amide group of protein, especially amide I, II and III regions [36,37]. The amide I (1700-1600 cm^{-1}), amide II (1582-1504 cm^{-1}) and III (1300-1200 cm^{-1}) were considered for indicating the film structure [38,39]. As shown in Fig. 3, native silk fibroin film (b) and the absorption peaks at 1680, 1557 and 1161 cm^{-1}. This suggested that the structure of silk fibroin was α-helix. The absorption peak of cellulose film (a) was mentioned at hydroxyl group (-OH) in range 3200-2900 cm^{-1} and methyl group (-CH) at 1550-1400 cm^{-1}. The SF/CE/SF composite film (c) showed the absorption peaks at 1666 cm^{-1}, low intensity of 1557 cm^{-1} peak and 1238 cm^{-1}, which revealed that the α-helix structure changed into random coil structure in higher ratio. This resulted to increase flexibility of the film. Beside the film, the CE/SF/CE composite film (d) showed higher intensity of the amide II peak (1538 cm^{-1}) and hydroxyl group (3500-3000 cm^{-1}). This might be concluded that the silk fibroin reinforced the formation of H-bond between amide and hydroxyl groups and resulted to increase β-structure ratio of the film.

Thermal studies: Thermal property of films considered from the weight loss after exposing to heat and rheological energy as shown in Fig. 4. The results indicated that all films have at least 3 decomposition points. The first is the temperature less than 100 ºC. This was due to the water evaporation and the second point of decomposition was in range of 250-300 ºC. This region involved the breakdown of hydrogen bonds between the chain of polymers and peptide bonds of silk protein structure [40]. The decomposition peaks are clearly observed from the DTG curves as shown in Fig. 5. The results indicated that the maximum temperature of decomposition rate ($T_{d,max}$) varied by the different films. The highest $T_{d,max}$ was found in the cellulose film, followed by CE/SF/CE, SF/CE/SF composite and the silk fibroin film, respectively. The composite films have the mixture $T_{d,max}$ of cellulose and silk fibroin. This confirmed that the CE/SF composite films were successfully prepared.

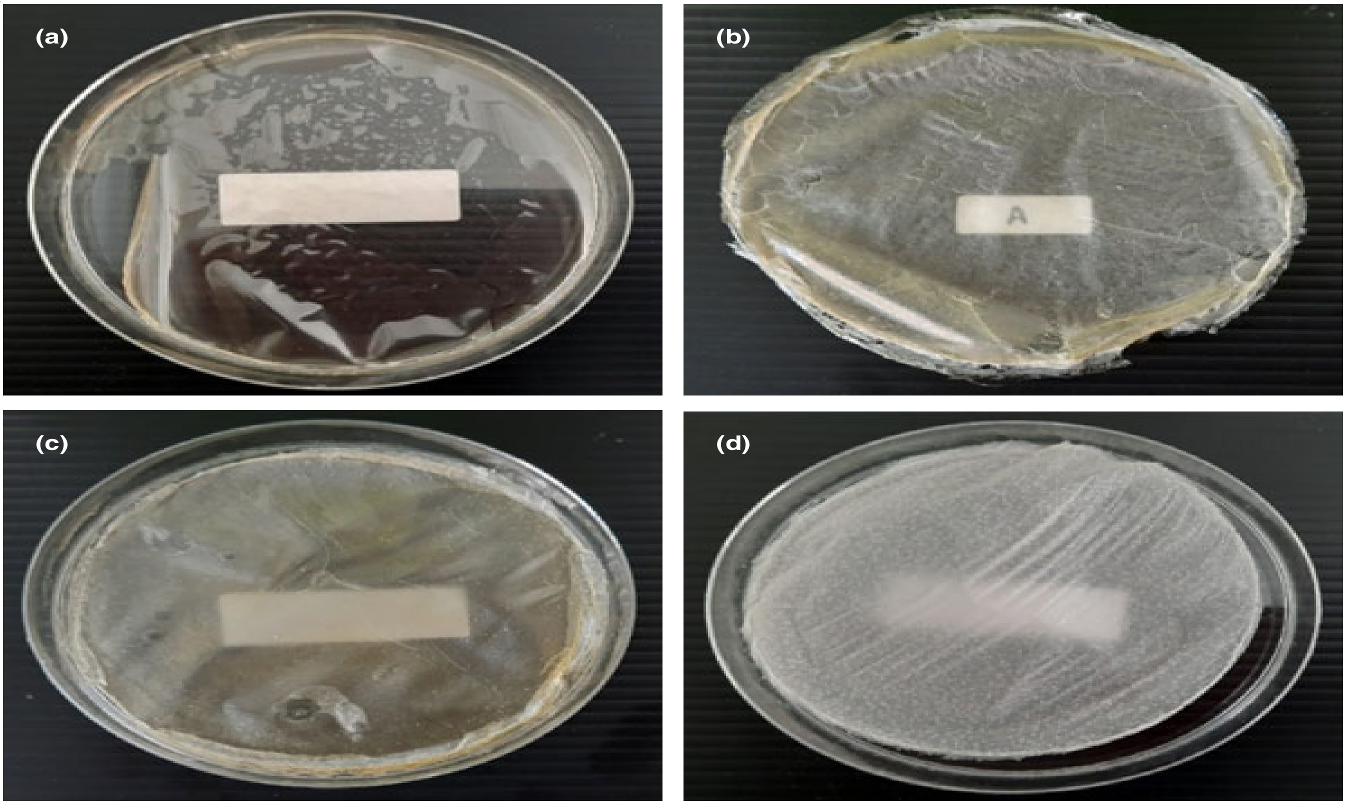

Fig. 1. Digital images of films transparency; SF (a), SF/CE/SF (b), CE/SF/CE (c) multilayers and CE (d) films

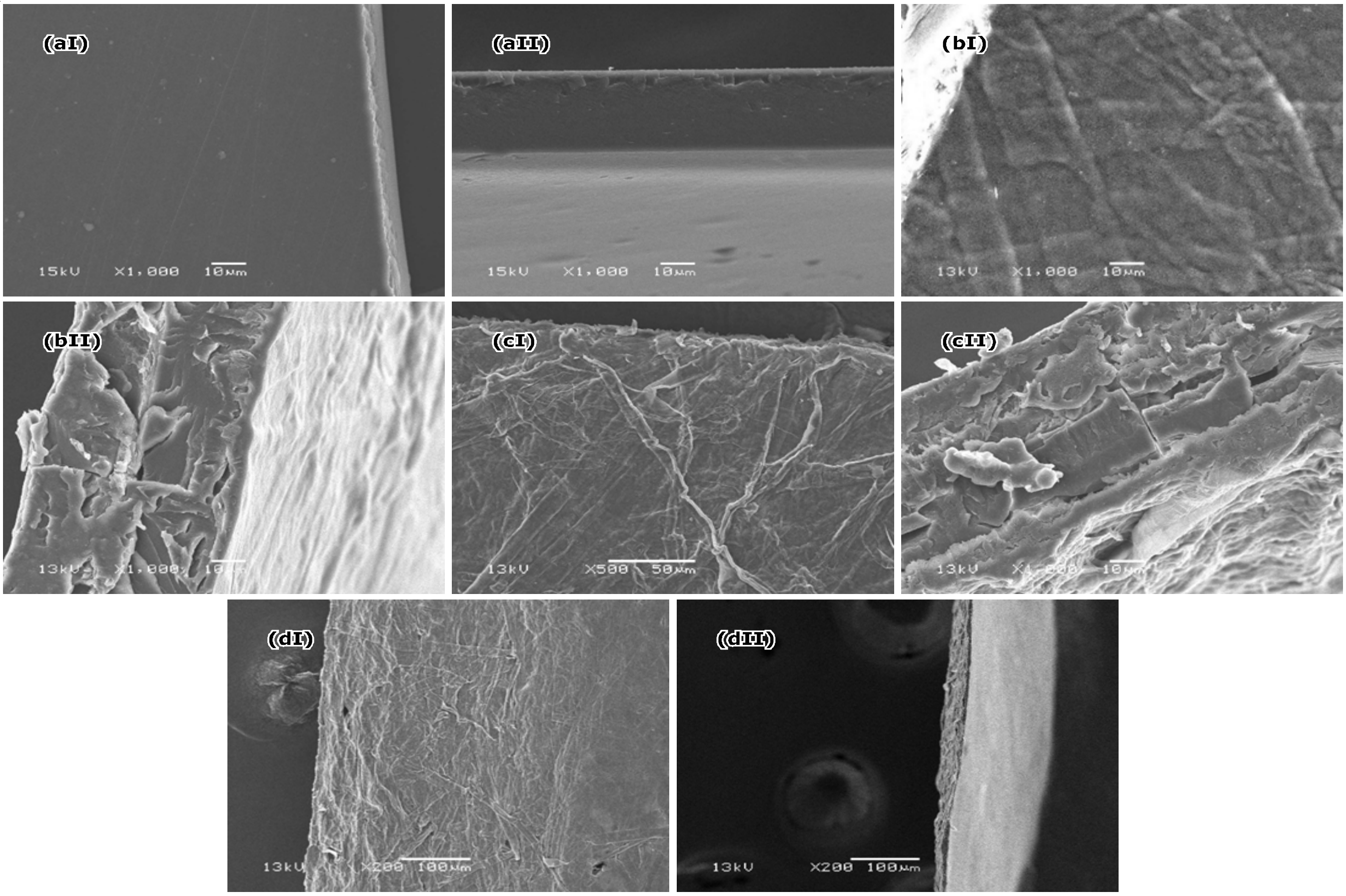

Fig. 2. SEM micrographs of different films; SF (a), multilayers SF/CE/SF (b), CE/SF/CE (c) and CE (d) films at 1,000X magnifications. I present as film surface and II as cross-section

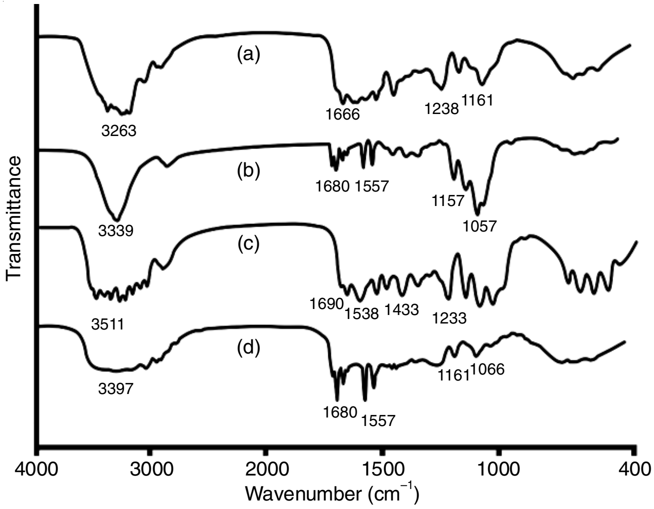

Fig. 3. FTIR spectra of different films; CE (a), SF (b), SF/CE/SF (c), CE/SF/CE (d) composite films

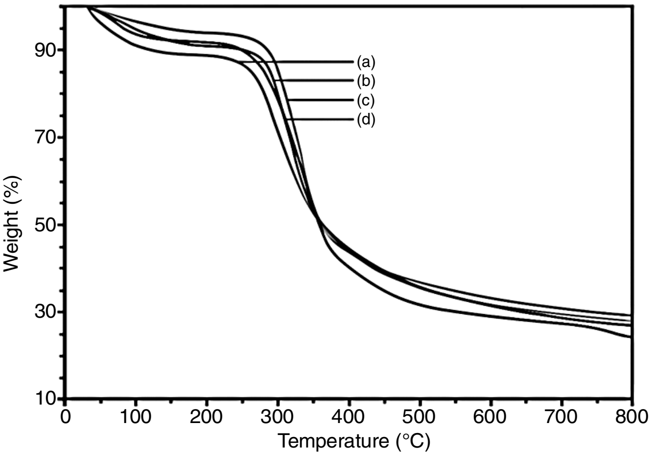

Fig. 4. TG curves of different films

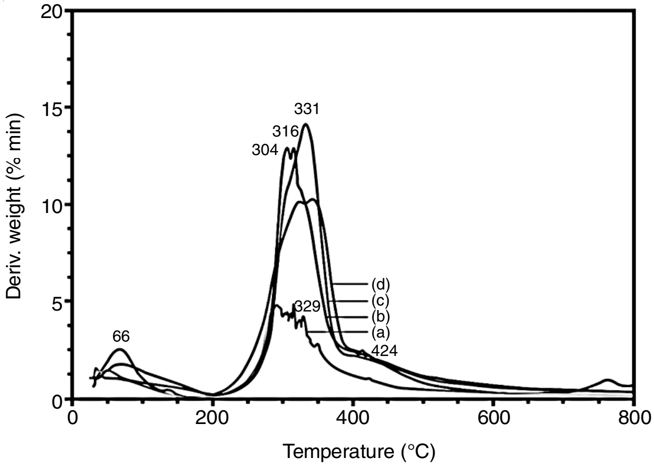

Fig. 5. DTG curves of different films

Water solubilty: The prepared films were treated in water for 7 days and the results are shown in Fig. 6. All the films rapidly dissolved at the initial time of treatment and then gradually increased until the end of testing. The cellulose film has the lowest percentage of water solubility while the silk fibroin film has the highest value. However, all tested films have percentage of water solubility lower than 12 even at 7 days of treatment. Among the composite films, the SF/CE/SF composite film has higher water solubility percentage than the CE/SF/CE film. Indeed, both cellulose and silk fibroin are non-degradable materials in water. Their structure is compact and dense texture by interaction bonds. However, the process of solution preparing by chemicals resulted to break the interaction bonds. The regenerated films of cellulose and silk fibroin from the solution state would be dissolved with hydrophilic molecule like water.

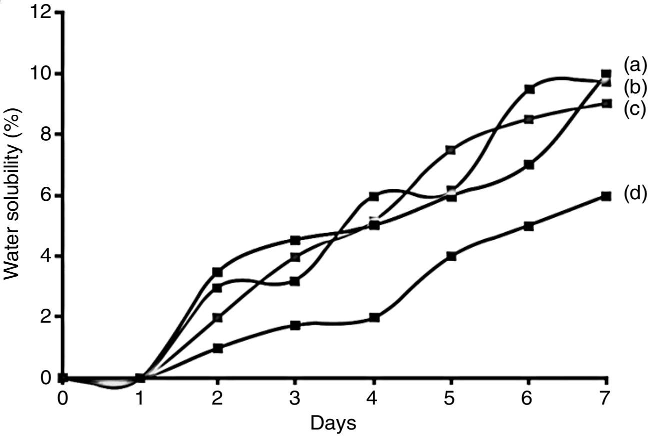

Fig. 6. Water solubility percentage of the different films

Conclusion

Cellulose solution from water hyacinth could be prepared and used as substrate for blending with silk fibroin as composite films. The transparency, morphology, secondary structure, thermal property as well as water solubility of the prepared films were varied by the unique structure and composition of the native polymers. Blending with cellulose helped to increase the flexibility of the composite films which observed by the ratio of random coil structure in the composite film. Moreover, cellulose could be increased the $T_{d,max}$ of silk fibroin film. The obtained results could be used as basic information for improvement the silk fibroin film properties as well as the wide applications of the water hyacinth cellulose as natural material.

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CONFLICT OF INTEREST

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

REFERENCES

1. M. Brodin, M. Vallejos, M.T. Opedal, M.C. Area and G. Chinga-Carrasco, *J. Clean. Prod.*, **162**, 646 (2017); https://doi.org/10.1016/j.jclepro.2017.05.209
2. V. Bertolino, G. Cavallaro, G. Lazzara, M. Merli, S. Milioto, F. Parisi and L. Sciascia, *Ind. Eng. Chem. Res.*, **55**, 7373 (2016); https://doi.org/10.1021/acs.iecr.6b01816

3. D. Kai, M.J. Tan, P.L. Chee, Y.K. Chua, Y.L. Yap and X.J. Loh, *Green Chem.*, **18**, 1175 (2016); https://doi.org/10.1039/C5GC02616D
4. H. Rajabinejad, M. Zoccola, A. Patrucco, A. Montarsolo, G. Rovero and C. Tonin, *Text. Res. J.*, **88**, 2415 (2018); https://doi.org/10.1177/0040517517723028
5. D. Sagnelli, K.H. Hebelstrup, E. Leroy, A. Rolland-Sabaté, S. Guilois, J.J.K. Kirkensgaard, K. Mortensen, D. Lourdin and A. Blennow, *Carbohydr. Polym.*, **152**, 398 (2016); https://doi.org/10.1016/j.carbpol.2016.07.039
6. J.G. Rouse and M.E. Van-Dyke, *Materials (Basel)*, **3**, 999 (2010); https://doi.org/10.3390/ma3020999
7. P.B. Dennis, A.Y. Walker, M.B. Dickerson, D.L. Kaplan and R.R. Naik, *Biomacromolecules*, **13**, 2037 (2012); https://doi.org/10.1021/bm300358g
8. E.M. Pritchard, P.B. Dennis, F. Omenetto, R.R. Naik and D.L. Kaplan, *Biopolymers*, **97**, 479 (2012); https://doi.org/10.1002/bip.22026
9. Q. Lu, X. Wang, X. Hu, P. Cebe, F. Omenetto and D.L. Kaplan, *Macromol. Biosci.*, **10**, 359 (2010); https://doi.org/10.1002/mabi.200900388
10. A.B. Li, J.A. Kluge, N.A. Guziewicz, F.G. Omenetto and D.L. Kaplan, *J. Control. Rel.*, **219**, 416 (2015); https://doi.org/10.1016/j.jconrel.2015.09.037
11. M. Gholipourmalekabadi, S. Sapru, A. Samadikuchaksaraei, R. Reis, D.L. Kaplan and S.C. Kundu, *Adv. Drug Deliv. Rev.*, **153**, 28 (2020); https://doi.org/10.1016/j.addr.2019.09.003
12. L.D. Koh, Y. Cheng, C.P. Teng, Y.W. Khin, X.J. Loh, S.Y. Tee, M. Low, E. Ye, H.D. Yu, Y.W. Zhang and M.-Y. Han, *Prog. Polym. Sci.*, **46**, 86 (2015); https://doi.org/10.1016/j.progpolymsci.2015.02.001
13. C. Vepari and D.L. Kaplan, *Prog. Polym. Sci.*, **32**, 991 (2007); https://doi.org/10.1016/j.progpolymsci.2007.05.013
14. A. Leal-Egana and T. Scheibel, *Biotechnol. Appl. Biochem.*, **55**, 155 (2010); https://doi.org/10.1042/BA20090229
15. D.N. Rockwood, R.C. Preda, T. Yucel, X. Wang, M.L. Lovett and D.L. Kaplan, *Nat. Protoc.*, **6**, 1612 (2011); https://doi.org/10.1038/nprot.2011.379
16. M.A. Tomeh, R. Hadianamrei and X.B. Zhao, *Pharmaceutics*, **11**, 429 (2019); https://doi.org/10.3390/pharmaceutics11100494
17. H. Wang, S. Duan, M. Wang, S. Wei, Y. Chen, W. Chen, Y. Li and S. Ding, *Sens. Actuators B Chem.*, **320**, 128376 (2020); https://doi.org/10.1016/j.snb.2020.128376
18. M.S. Lee, C.-S. Hung, D.A. Phillips, C.C. Buck, M.K. Gupta and M.W. Lux, *Synth. Syst. Biotechnol.*, **5**, 145 (2020); https://doi.org/10.1016/j.synbio.2020.06.004
19. C.Y. Wang, K.L. Xia, Y.Y. Zhang and D.L. Kaplan, *Acc. Chem. Res.*, **52**, 2916 (2019); https://doi.org/10.1021/acs.accounts.9b00333
20. R.K. Pal, A.A. Farghaly, M.M. Collinson, S.C. Kundu and V.K. Yadavalli, *Adv. Mater.*, **28**, 1406 (2016); https://doi.org/10.1002/adma.201504736
21. L. Cheng, J. Shao, F. Wang, Z. Li and F. Dai, *Mater. Des.*, **195**, 108988 (2020); https://doi.org/10.1016/j.matdes.2020.108988
21. Y. Guan, H. You, J. Cai, Q. Zhang, S. Yan and R. You, *Carbohydr. Polym.*, **239**, 116232 (2020); https://doi.org/10.1016/j.carbpol.2020.116232
22. Y. Feng, X. Li, Q. Zhang, S. Yan, Y. Guo, M. Li and R. You, *Carbohydr. Polym.*, **216**, 17 (2019); https://doi.org/10.1016/j.carbpol.2019.04.008
23. X. Hu, P. Cebe, A.S. Weiss, F. Omenetto and D.L. Kaplan, *Mater. Today*, **15**, 208 (2012); https://doi.org/10.1016/S1369-7021(12)70091-3
24. R. You, J. Zhang, S. Gu, Y. Zhou, X. Li, D. Ye and W. Xu, *Mater. Sci. Eng. C*, **79**, 430 (2017); https://doi.org/10.1016/j.msec.2017.05.063
25. M. Ul-Islam, T. Khan and J.K. Park, *Carbohydr. Polym.*, **88**, 596 (2012); https://doi.org/10.1016/j.carbpol.2012.01.006
26. J.S. Lee, B. Parameswaran, J.P. Lee and S.C. Park, *J. Sci. Ind. Res. (India)*, **67**, 865 (2008).
27. D. Klemm, B. Heublein, H.P. Fink and A. Bohn, *Angew. Chem. Int. Ed.*, **44**, 3358 (2005); https://doi.org/10.1002/anie.200460587
28. J. Shokri and K. Adibkia, Application of Cellulose and Cellulose Derivatives in Pharmaceutical Industries, In: Cellulose-Medical, Pharmaceutical and Electronic Applications, In Tech, pp. 47 (2013).
29. S. Singh, V.K. Singh, M. Aamir, M.K. Dubey, J.S. Patel, R.S. Upadhyay and V.K. Gupta, eds.: V. Gupta, Cellulase in Pulp and Paper Industry, In: New and Future Developments in Microbial Biotechnology and Bioengineering, pp. 152-162 (2016).
30. B. Peng, Z. Yao, X. Wang, M. Crombeen, D.G. Sweeney and K.C. Tam, *Green Energy Environ.*, **5**, 37 (2020); https://doi.org/10.1016/j.gee.2019.09.003
31. S. Chakraborty, R. Gupta, K.K. Jain, Hemanshi, S. Gautam and R.C. Kuhad, eds.: V. Gupta, Cellulases: Application in Wine and Brewery Industry, In: New and Future Developments in Microbial Biotechnology and Bioengineering, Elsevier, pp. 193-200 (2016).
32. A.K. Bledzki and J. Gassan, *Prog. Polym. Sci.*, **24**, 221 (1999); https://doi.org/10.1016/S0079-6700(98)00018-5
33. H. Wissel, C. Mayr and A. Lücke, *Org. Geochem.*, **39**, 1545 (2008); https://doi.org/10.1016/j.orggeochem.2008.07.014
34. G.N. Juárez-Luna, E. Favela-Torres, I.R. Quevedo and N. Batina, *Carbohydr. Polym.*, **220**, 110 (2019); https://doi.org/10.1016/j.carbpol.2019.05.058
35. Y.L. Zhu, A.M. Zayed, J.-H. Qian, M. Souza and N. Terry, *J. Environ. Qual.*, **28**, 339 (1999); https://doi.org/10.2134/jeq1999.00472425002800010042x
36. A. Aluigi, C. Vineis, A. Varesano, G. Mazzuchetti, F. Ferrero and C. Tonin, *Eur. Polym. J.*, **44**, 2465 (2008); https://doi.org/10.1016/j.eurpolymj.2008.06.004
37. N. Ramakrishnan, S. Sharma, A. Gupta and B.Y. Alashwal, *Int. J. Biol. Macromol.*, **111**, 352 (2018); https://doi.org/10.1016/j.ijbiomac.2018.01.037
38. D.C. Castrillón Martínez, C.L. Zuluaga, A. Restrepo-Osorio and C. Álvarez-López, *Procedia Eng.*, **200**, 377 (2017); https://doi.org/10.1016/j.proeng.2017.07.053
39. P. Shi and J.C.H. Goh, *Powder Technol.*, **215-216**, 85 (2012); https://doi.org/10.1016/j.powtec.2011.09.012
40. X. Zhang, J. Feng, X. Liu and J. Zhu, *Carbohydr. Polym.*, **89**, 67 (2012); https://doi.org/10.1016/j.carbpol.2012.02.047