

## REFERENCES

- Al-khalifawi, I. & Hassan, I. A. (2014). Factors Influence on the yield of Bacterial Cellulose of Kombucha (Khubdat Humza). *Baghdad Science Journal*, 11(3), 1420-1428.
- Bae, S., & Shoda, M. (2004). Bacterial cellulose production by fed-batch fermentation in molasses medium. *Biotechnology Progress*, 20(5), 1366–1371. <http://doi.org/10.1021/bp0498490>
- Brown, Adrian J. (1886). An Acetic Ferment Which Forms Cellulose. *Chemical Society* 49, 432–439.
- Carreira, P., Mendes, J., A., Trovatti, E., Serafim, L., S., Freire, C., S., Silvestre, A., J., & Neto, C., P. (2011). Utilization of residues from agro-forest industries in the production of high value bacterial cellulose. *Bioresource Technol*, 102(15):7354–7360.
- Çakar, F, Özer, I., Aytekin, A.Ö., & Şahin, F. (2014). Improvement Production of Bacterial Cellulose by Semi-Continuous process in Molasses Medium. *Carbohydrate Polymers* 106, 7–13.
- Chakravorty, S., Bhattacharya, S., Chatzinotas, A., Chakraborty, W., Bhattacharya, D., & Gachhui, R. (2016). Kombucha tea fermentation: Microbial and biochemical dynamics. *International Journal of Food Microbiology*, 220, 63–72.
- Chawla, P. R., Bajaj, I. B., Survase, S. A., & Singhal, R. S. (2009). bacterial cellulose: Fermentative production and applications. *Food Technology and Biotechnology*, 47(2), 107–124.
- Chen, S. Q., Lopez-Sanchez, P., Wang, D., Mikkelsen, D., & Gidley, M. J. (2018). Mechanical properties of bacterial cellulose synthesised by diverse strains of the genus Komagataeibacter. *Food Hydrocolloids*, 81, 87–95. <https://doi.org/10.1016/j.foodhyd.2018.02.031>
- Costa, A., Almeida, F., Vinhas, G., & Sarubbo, L. (2017). Production of Bacterial Cellulose by Using Corn Steep Liquor As Nutrient Sources. *Frontiers in Microbiology*, 8, 2027. <https://doi.org/10.3389/fmicb.2017.02027>
- Dirisu, C., Rosenzweig, J., Lambert, E., & Oduah, A. (2017). pH Effect and pH Changes during Biocellulose Production by Gluconacetobacter xylinus in Moringa oleifera Tea-Sugar Medium. *Journal of Advances in Microbiology*, 7(2), 1–7. <http://doi.org/10.9734/JAMB/2017/38440>
- Dufresne, A. (2017). Bacterial Cellulose. In *Nanocellulose: From Nature to High Performance Tailored Materials*(pp. 125-142). Berlin: De Gruyter.
- Drury, J. L., Dennis, R. G., & Mooney, D. J. (2004). The tensile properties of alginate hydrogels. *Biomaterials*, 25(16), 3187–3199. <https://doi.org/10.1016/j.biomaterials.2003.10.002>
- Embuscado, M. E., Marks, J. S., & BeMiller, J. N. (1994). Bacterial cellulose. I. Factors affecting the production of cellulose by Acetobacter xylinum. *Food Hydrocolloids* 8: 407-418
- Esa, F., Tasirin, S. M., & Rahman, N. A. (2014). Overview of Bacterial Cellulose Production and Application. *Agriculture and Agricultural Science Procedia*, 2, 113–119. <http://doi.org/10.1016/j.aaspro.2014.11.017>
- Faridah, F., Diana, S., Helmi, H., Sami, M., & Mudliana, M. (2013). Effect of Sugar Concentrations on Bacterial Cellulose Production as Cellulose Membrane in Mixture Liquid Medium and Material Properties Analysis. *ASEAN/Asian Academic Society International Conference Proceeding Series*, 96–101.
- Fontana, J. D., Franco, V. C., Souza, S. J., Lyra, I. N., & Souza, A. M. (1991). Nature of plant stimulators in the production of Acetobacter xylinum (“tea fungus”) biofilm used in skin therapy. *Applied Biochemistry and Biotechnology*, 28-29(1), 341-351. doi:10.1007/bf02922613
- Goh, W. N., Rosma, A., Kaur, B., Fazilah, A., Karim, A. A., & Bhat, R. (2012). Fermentation of black tea broth (kombucha): I. effects of sucrose concentration and fermentation time on the yield of microbial cellulose. *International Food Research Journal*, 19(1), 109–117. <http://doi.org/10.1016/j.pec.2010.07.039>
- Greenwalt, C. J., Steinkraus, K. H., & Ledford, R. A. (2000). Kombucha, the Fermented Tea: Microbiology, Composition, and Claimed Health Effects. *Journal of Food Protection*, 63(7), 976-981. doi:10.4315/0362-028x-63.7.976

- Harper, C. A. (2006). *Handbook of plastics technologies the complete guide to properties and performance*. New York: McGraw-Hill.
- Hong, F., Zhu, Y., Yang, G., & Yang, X. (2011). Wheat straw acid hydrolysate as a potential cost-effective feedstock for production of bacterial cellulose. *J Chem Technol Biot*, 86(5):675–680.
- Huang, Y., Zhu, C., Yang, J., Nie, Y., Chen, C., & Sun, D. (2014). Recent advances in bacterial cellulose. *Cellulose*, 21(1), 1–30. <http://doi.org/10.1007/s10570-013-0088-z>
- Hwang, J. W., Yang, Y. K., Hwang, J. K., Pyun, Y. R., & Kim, Y. S. (1999). Effects of pH and dissolved oxygen on cellulose production by *Acetobacter xylinum* BRC5 in agitated culture. *Journal of Bioscience and Bioengineering*, 88(2), 183-188. doi:10.1016/s1389-1723(99)80199-6
- Indrianingsih, A. W., Rosyida, V. T., Jatmiko, T. H., Prasetyo, D. J., Poeloengasih, C. D., Apriyana, W., ... Ratih, D. (2017). Preliminary study on biosynthesis and characterization of bacteria cellulose films from coconut water. *IOP Conference Series: Earth and Environmental Science*, 101(1). <https://doi.org/10.1088/1755-1315/101/1/012010>
- Jaya A K, Mahendra A. (2008). Pra desain pabrik ethanol dari molasses. Retrieved from <https://www.slideshare.net/mah3ndr4/indonesia-mandiri>
- Jayabalan, R., Malbaša, R. V., Lončar, E. S., Vitas, J. S., & Sathishkumar, M. (2014). A review on kombucha tea-microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. *Comprehensive Reviews in Food Science and Food Safety*, 13(4), 538–550. <http://doi.org/10.1111/1541-4337.12073>
- Joris, K. F., Wulfand, B. P., & Vandamme, E. (1993). Enhanced bacterial cellulose yield in aerated *Acetobacter xylinum* culture by adding micro-particles. *Cellulosic Material for Selective Separation and Other Technology*, Kennedy, J. F., Philips, G. O. and William, P. A. ed. Ellis Horwood, London, 239-245.
- Jung, H.-I., Lee, O.-M., Jeong, J.-H., Jeon, Y.-D., Park, K.-H., Kim, H.-S., ... Son, H.-J. (2009). Production and Characterization of Cellulose by *Acetobacter* sp. V6 Using a Cost-Effective Molasses–Corn Steep Liquor Medium. *Applied Biochemistry and Biotechnology*, 162(2), 486–497. doi:10.1007/s12010-009-8759-9
- Kaedah, K., Berbeza, P., Nata, T., Pa'E, N., Hamid, N. I. A., Khairuddin, N., ... Muhamad, I. I. (2014). Effect of Different Drying Methods on the Morphology, Crystallinity, Swelling Ability and Tensile Properties of Nata De Coco. *Sains Malaysiana*, 43(5), 767–773. Retrieved from [http://journalarticle.ukm.my/7159/1/16\\_Norhayati\\_PaE.pdf](http://journalarticle.ukm.my/7159/1/16_Norhayati_PaE.pdf)
- Kallel, L., Desseaux, V., Hamdi, M., Stocker, P., & Ajandouz, E.H., (2012). Insights into the fermentation biochemistry of Kombucha teas and potential impacts of Kombucha drinking on starch digestion. *Food Res. Int.*, 49, 226–232.
- Keshk, S. M. a S., Razek, T. M. a, & Sameshima, K. (2006). Bacterial cellulose production from beet molasses. *Acta Biomater.*, 5(17), 1519–1523. <http://doi.org/10.3305/nh.2015.31.5.7418>
- Keshk, S., & Sameshima, K. (2006). The utilization of sugar cane molasses with/without the presence of lignosulfonate for the production of bacterial cellulose. *Applied Microbiology and Biotechnology*, 72(2), 291–296. <http://doi.org/10.1007/s00253-005-0265-6>
- Kuo, C., Chen, J., Liou, B., & Lee, C. (2016). Utilization of acetate buffer to improve bacterial cellulose production by *Gluconacetobacter xylinus*. *Food Hydrocolloids*, 53, 98-103. doi:10.1016/j.foodhyd.2014.12.034
- Krystynowicz, A., Czaja, W., Wiktorowska-Jezierska, A., Gonçalves-Miśkiewicz, M., Turkiewicz, M., & Bielecki, S. (2002). Factors affecting the yield and properties of bacterial cellulose. *Journal of Industrial Microbiology & Biotechnology*, 29(4), 189-195. doi:10.1038/sj/jim/7000303
- Lazaridou, A., Roukas, T., Biliaderis, C. ., & Vaikousi, H. (2002). *Characterization of pullulan produced from beet molasses by Aureobasidium pullulans in a stirred tank reactor under varying agitation*. *Enzyme and Microbial Technology*, 31(1-2), 122–132. doi:10.1016/s0141-0229(02)00082-0

- Lee, K. Y., Buldum, G., Mantalaris, A., & Bismarck, A. (2014). More than meets the eye in bacterial cellulose: Biosynthesis, bioprocessing, and applications in advanced fiber composites. *Macromolecular Bioscience*, 14(1), 10–32. <http://doi.org/10.1002/mabi.201300298>
- Liu, C., Hsu, W., Lee, F., & Liao, C. (1996). The isolation and identification of microbes from a fermented tea beverage, Haipao, and their interactions during Haipao fermentation. *Food Microbiology*, 13(6), 407-415. doi:10.1006/fmic.1996.0047
- Lu, H., & Jiang, X. (2014). Structure and properties of bacterial cellulose produced using a trickling bed reactor. *Applied Biochemistry and Biotechnology*, 172(8), 3844–3861. <https://doi.org/10.1007/s12010-014-0795-4>
- Malbaša, R., Lončar, E., & Djurić, M. (2007a). Comparison of the products of kombucha fermentation on sucrose and molasses. *Food Chemistry*, 106, 1039–1045
- Malbaša, R., Lončar, E., Djurić, M., & Došenović, I. (2007b). Effect of sucrose concentration on the products of Kombucha fermentation on molasses. *Food Chemistry*, 108(3), 926-932. doi:10.1016/j.foodchem.2007.11.069
- Matsuoka, M., Tsuchida, T., Matsushita, K., Adachi, O., & Yoshinaga, F. (1996). A synthetic medium for bacterial cellulose production by *Acetobacter xylinum* subsp. *sucrofermentans*. *Biosci. Biotechnol. Biochem*, 60, 575–579.
- Marsh, A. J., Osullivan, O., Hill, C., Ross, R. P., & Cotter, P. D. (2014). Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungus) samples. *Food Microbiology*, 38, 171-178. doi:10.1016/j.fm.2013.09.003
- Mikkelsen, D., Flanagan, B. M., Dykes, G. A., & Gidley, M. J. (2009). Influence of different carbon sources on bacterial cellulose production by *Gluconacetobacter xylinus* strain ATCC 53524. *Journal of Applied Biology*, 107, 576–583. <https://doi.org/10.1111/j.1365-2672.2009.04226.x>
- Mohammed, M.J., & Al-Bayati, F.A. (2009). Isolation, identification and purification of caffeine from *Coffea Arabica* L. and *Camellia sinensis* L.: A combination antibacterial study. *International Journal of Green Pharmacy*, 3(1), 52-57
- Moosavi-nasab, M., & Yousefi, A. R. (2010). Investigation of Physicochemical Properties of the Bacterial Cellulose Produced by *Gluconacetobacter xylinus* from Date Syrup. *Cellulose*, 4(8), 1248–1253.
- Muhamad, I. I., Zahan, K. A., & Pa'e, N. (2017). Bacterial Cellulose as Secondary Metabolite: Production, Processing, and Applications. In *Plant Secondary Metabolites*(pp. 171-196). Oakville, ON: Apple Academic Press.
- Nair, K. G. (1966). Purification and Properties of 3',5'-Cyclic Nucleotide Phosphodiesterase from Dog Heart\*. *Biochemistry*, 5(1), 150–157. doi:10.1021/bi00865a020
- Naritomi, T., Kouda, T., Yano, H., & Yoshinaga, F. (1998). Effect of ethanol on bacterial cellulose production from fructose in continuous culture. *Journal of Fermentation and Bioengineering*, 85(6), 598-603. doi:10.1016/s0922-338x(98)80012-3
- Nguyen, V. T., Flanagan, B., Gidley, M. J., & Dykes, G. A. (2008). Characterization of cellulose production by a *Gluconacetobacter xylinus* strain from Kombucha. *Current Microbiology*, 57, 449–453. <http://doi.org/10.1007/s00284-008-9228-3>
- Nonthakaew, A., Matan, N., Aewsiri, T., & Matan, N. (2015). Caffeine in foods and its antimicrobial activity. *International Food Research Journal*, 22(1), 9–14.
- Phisalaphong, M. & Jatupaiboon, N. (2009). Biosynthesis and characterization of bacterial cellulose-chitosan film. *Carbohydr. Polym*, 74, 482–488.
- Pourramezan, G., Roayaei, A., & Qezelbash, Q. (2009). Optimization of Culture Conditions for Bacterial Cellulose Production by *Acetobacter* sp. 4B-2. *Biotechnology(Faisalabad)*, 8(1), 150-154. doi:10.3923/biotech.2009.150.154
- Portela, R., Leal, C. R., Almeida, P. L., & Sobral, R. G. (2019). Bacterial cellulose: a versatile biopolymer for wound dressing applications. *Microbial Biotechnology*, 2019. <https://doi.org/10.1111/1751-7915.13392>

- Premjet, S., Premjet, D., & Ohtani, Y. (2007). The Effect of Ingredients of Sugar Cane Molasses on Bacterial Cellulose Production by *Acetobacter xylinum* ATCC 10245. *Fiber*, 63(8), 193-199. doi:10.2115/fiber.63.193
- Raghunathan, D. (2013). Production of Microbial Cellulose from the New Bacterial Strain Isolated From Temple Wash Waters. *Int.J.Curr.Microbiol.App.Sci*, 2(12), 275–290. <http://doi.org/2004R0726-v.7> of 05.06.2013
- Rahman, Gomaa, N. A., Nassar, Nadia, R. A., Heikal, Yehia, A., Donia, Mohmoud, A. M. A., Naguib, Mohamed, M., & Fadel, M. (2016). Effect of Different Treatments on Heavy Metal Concentration in Sugar Cane Molasses. *International Journal of Agricultural and Biosystems Engineering*, 10(1), 43–48.
- Ramana, K., V., Tomar, A., & Singh, L. (2000). Effect of various carbon and nitrogen sources on cellulose synthesis by *Acetobacter xylinum*. *World J. Microbiol. Biotechnol*, 16, 245–248.
- Ryan, R. P., Fouhy, Y., Lucey, J. F., & Dow, J. M. (2006). Cyclic Di-GMP Signaling in Bacteria: Recent Advances and New Puzzles. *Journal of Bacteriology*, 188(24), 8327-8334. doi:10.1128/jb.01079-06
- Savitri Kumar, N., Hewavitharange, P., & Adikaram, N.K.B. (1995). Attack on tea by *Xyleborus fornicatus*: Inhibition of the symbiote, *Monacrosporium ambrosium* by caffeine. *Phytochemistry*, 40(4), 1113- 1116
- Shezad, O., Khan, S., Khan, T., & Park, J. K. (2010). Physicochemical and mechanical characterization of bacterial cellulose produced with an excellent productivity in static conditions using a simple fed-batch cultivation strategy. *Carbohydrate Polymers*, 82(1), 173–180. <https://doi.org/10.1016/j.carbpol.2010.04.052>
- Singh, O., Panesar, P. S., & Chopra, H. K. (2017). *Response surface optimization for cellulose production from agro industrial waste by using new bacterial isolate Gluconacetobacter xylinus C18*. *Food Science and Biotechnology*, 26(4), 1019–1028. doi:10.1007/s10068-017-0143-x
- Son, H.-J., Heo, M.-S., Kim, Y.-G., & Lee, S.-J. (2001). Optimization of fermentation conditions for the production of bacterial cellulose by a newly isolated *Acetobacter* sp.A9 in shaking cultures. *Biotechnology and Applied Biochemistry*, 33(1), 1. <http://doi.org/10.1042/BA20000065>
- Tantratian, S., Tammarate, P., Krusong, W., Bhattarakosol, P., & Phunsri, A. (2005). Effect of Dissolved Oxygen on Cellulose Production by *Acetobacter* sp. *J. Sci. Res. Chula. Univ.*, 30(2), 179–186.
- Teoh, A. L., Heard, G., & Cox, J. (2004). Yeast ecology of Kombucha fermentation. *International Journal of Food Microbiology*, 95(2), 119–126. <http://doi.org/10.1016/j.ijfoodmicro.2003.12.020>
- Tsouko, E., Kourmentza, C., Ladakis, D., Kopsahelis, N., Mandala, I., Papanikolaou, S., . . . Koutinas, A. (2015). Bacterial Cellulose Production from Industrial Waste and by-Product Streams. *International Journal of Molecular Sciences*, 16(12), 14832-14849. doi:10.3390/ijms160714832
- Tyagi, N., & Suresh, S. (2016). Production of cellulose from sugarcane molasses using *Gluconacetobacter intermedius* SNT-1: Optimization & characterization. *Journal of Cleaner Production*, 112, 71-80. doi:10.1016/j.jclepro.2015.07.054
- Velmurugan, P., Myung, H., Govarthanan, M., Yi, Y. J., Seo, S. K., Cho, K. M., ... Oh, B. T. (2015). Production and characterization of bacterial cellulose by *Leifsonia* sp. CBNU-EW3 isolated from the earthworm, *Eisenia fetida*. *Biotechnology and Bioprocess Engineering*, 20(3), 410–416. <http://doi.org/10.1007/s12257-014-0793-y>
- Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J., & Taillandier, P. (2018). Understanding Kombucha Tea Fermentation: A Review. *Journal of Food Science*, 83(3), 580-588. doi:10.1111/1750-3841.14068
- Wanichapichart, P., Kaewnopparat, S., Buaking, K., Puthai, W. (2002). Characterization of cellulose membranes produced by *Acetobacter xylinum*. *J. Sci. Technol.*, 24, 855–862
- Watanabe, K., Tabuchi, M., Morinaga, Y., & Yoshinaga, F. (1998). Structural features and properties of bacterial cellulose produced in agitated culture. *Cellulose*, 5(3), 187–200. <https://doi.org/10.1023/A:1009272904582>

- Watawana, M. I., Jayawardena, N., Gunawardhana, C. B., & Waisundara, V. Y. (2015). Enhancement of the antioxidant and starch hydrolase inhibitory activities of king coconut water (*Cocos nuciferavar. aurantiaca*) by fermentation with kombucha 'tea fungus'. *International Journal of Food Science & Technology*, *51*(2), 490-498. doi:10.1111/ijfs.13006
- Yeoh Q. L., Lee G. L., & Fatimah, H. (1985). Teknologi Pengeluaran Nata. *J. Teknologi Makanan*, *4*(1), 36-39.
- Yodsuwan, N., Owatworakit, A., Ngaokla, A., & Tawichai, N. (2012). Effect of carbon and nitrogen sources on bacterial cellulose production for bionanocomposite materials.
- Yoshino, T., Asakura, T., & Toda, K. (1996). Cellulose production by *Acetobacter pasteurianus* on silicone membrane. *J. Ferment. Bioeng*, *81*, 32-36.
- Zahan, K. A., Nordin, K., Mustapha, M., & Zairi, M. N. (2015). Effect of Incubation Temperature on Growth of *Acetobacter xylinum* 0416 and Bacterial Cellulose Production. *Applied Mechanics and Materials*, *815*, 3-8. doi:10.4028/www.scientific.net/amm.815.3
- Zahan, K. A., Pa'e, N., & Muhamad, I. I. (2015). Monitoring the Effect of pH on Bacterial Cellulose Production and *Acetobacter xylinum* 0416 Growth in a Rotary Discs Reactor. *Arabian Journal for Science and Engineering*, *40*(7), 1881-1885. <http://doi.org/10.1007/s13369-015-1712-z>

## APPENDICES

### Appendix 1. Composition of Fermentation Medium of Each Step of Starter Culture Preparation

	Step 1	Step 2	Step 3
Black tea (g)	0.5	2	-
Table sugar (g)	5	20	-
Molasses (g)	-	20	100
Total Volume (ml)	50	200	1000
Water (ml)	45	180	-
200mM acetate buffer pH 4.75 (ml)	-	-	900
Culture (ml)	5 <sup>a</sup>	20 <sup>b</sup>	100 <sup>c</sup>

### Appendix 2. Composition of Fermentation Medium of Each Setups

		Molasses (g)	Caffeine (mg)	200 mM acetate buffer (ml)			Water (ml)	Culture (ml)
				pH 4.0	pH 4.75	pH 5.5		
Molasses Concentration (w/v)	Level 1	50	250	-	450	-	-	50
	Level 2	75	250	-	450	-	-	50
	Level 3	100	250	-	450	-	-	50
Caffeine Concentration (mg/L)	Control	75	0	-	450	-	-	50
	Level 1	75	150	-	450	-	-	50
	Level 2	75	250	-	450	-	-	50
	Level 3	75	350	-	450	-	-	50
pH of 200 mM Acetate Buffer	Control	75	250	-	-	-	450	50
	Level 1	75	250	450	-	-	-	50
	Level 2	75	250	-	450	-	-	50
	Level 3	75	250	-	-	450	-	50

**Appendix 3. Result of Experiment 1: Effect of Different Initial Molasses Concentration on the Yield and the Properties of Bacterial Cellulose Biofilms**



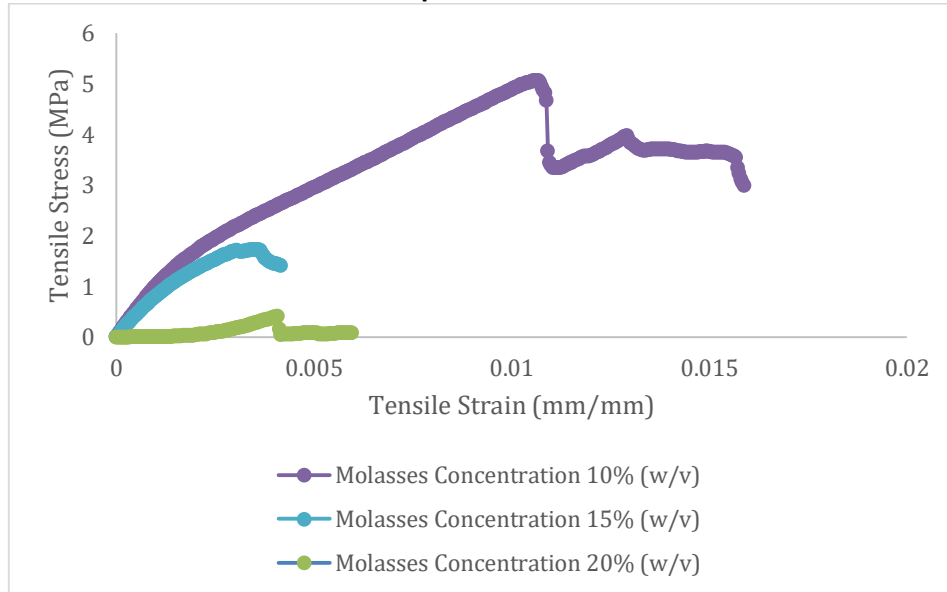
**Appendix 3.1. Physical appearance of BC biofilms produced at different initial molasses concentration.** From left to right: produced at 10%, 15%, and 20% (w/v) molasses concentration

**Appendix 3.2. Result of Experiment 1**

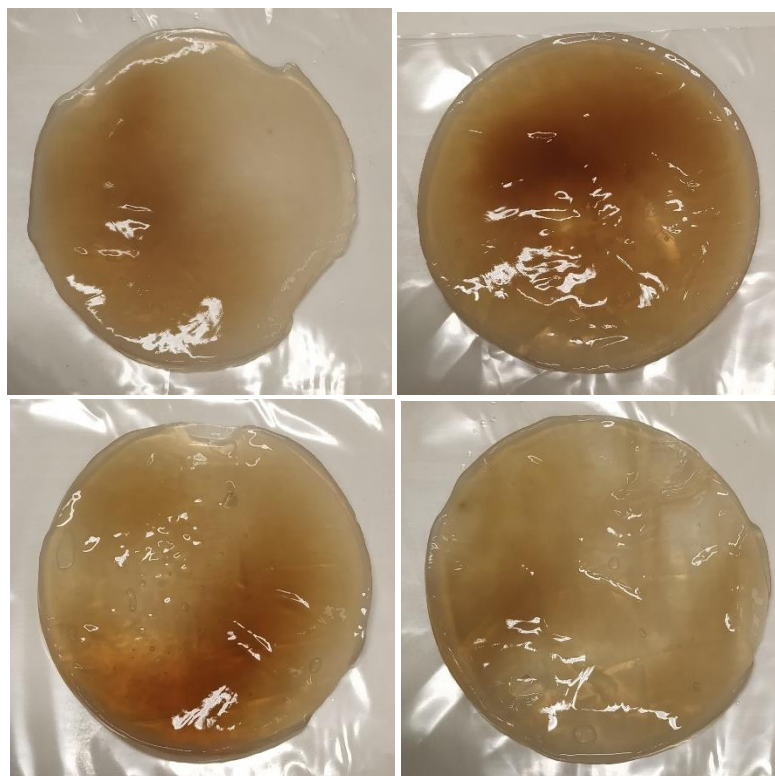
	Molasses Concentration (w/v)		
	10%	15%	20%
BC Yield (g/L)	1.32±0.30 <sup>a</sup>	1.29±0.45 <sup>a</sup>	0.36±0.07 <sup>a</sup>
Tensile Strength (MPa)	24.91±0.96 <sup>a</sup>	14.22±2.21 <sup>b</sup>	3.00±0.66 <sup>c</sup>
Elongation Percentage (%)	1.02±0.35 <sup>a</sup>	1.42±0.38 <sup>b</sup>	0.29±0.10 <sup>b</sup>
Young's Modulus (MPa)	1205.6±40.86 <sup>a</sup>	766.99±26.53 <sup>b</sup>	-
Water Holding Capacity (%)	297.9±29.6 <sup>a</sup>	366.1±56.6 <sup>a</sup>	233.9±37.8 <sup>a</sup>
Initial pH	4.75±0.02	4.74±0.02	4.74±0.03
Final pH	4.31±0.05	4.25±0.01	4.27±0.05

<sup>a,b,c</sup> Means in the same row bearing different superscript letters differ significantly ( $P < 0.05$ ). Values represent means  $\pm$  standard error of quadruplicate experiments.

### Appendix 3.3. Stress-strain Curve of Samples Produced at Different Molasses Concentration



### Appendix 4. Result of Experiment 2: Effect of Different Initial Caffeine Concentration on the Yield and the Properties of Bacterial Cellulose Biofilms



#### Appendix 4.1. Physical Appearance of BC Produced at Different Caffeine Concentration

First row, from left to right: produced at 0 mg/L and 300 mg/L caffeine concentration  
Second row, from left to right: produced at 500 and 700 mg/L caffeine concentration

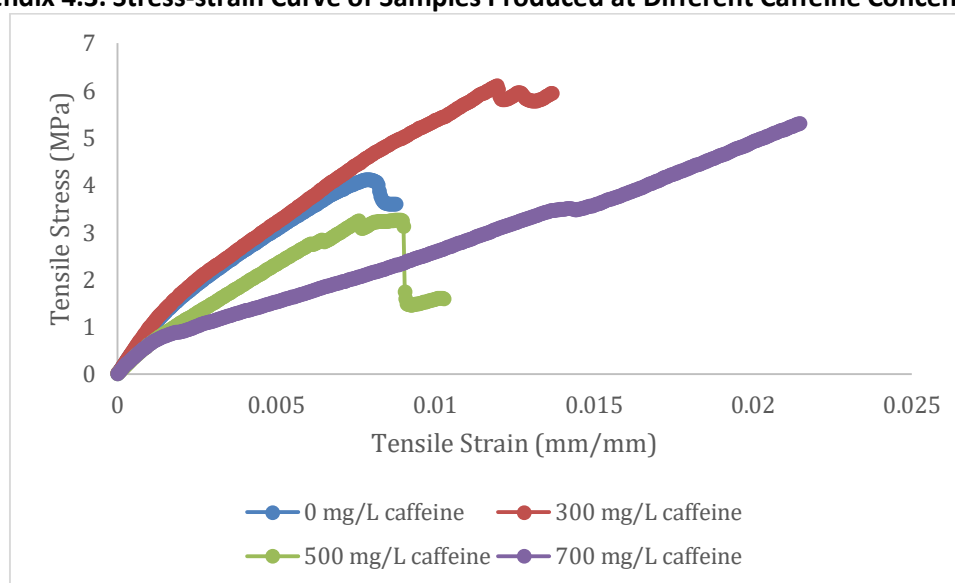


### Appendix 4.2. Result of Experiment 2

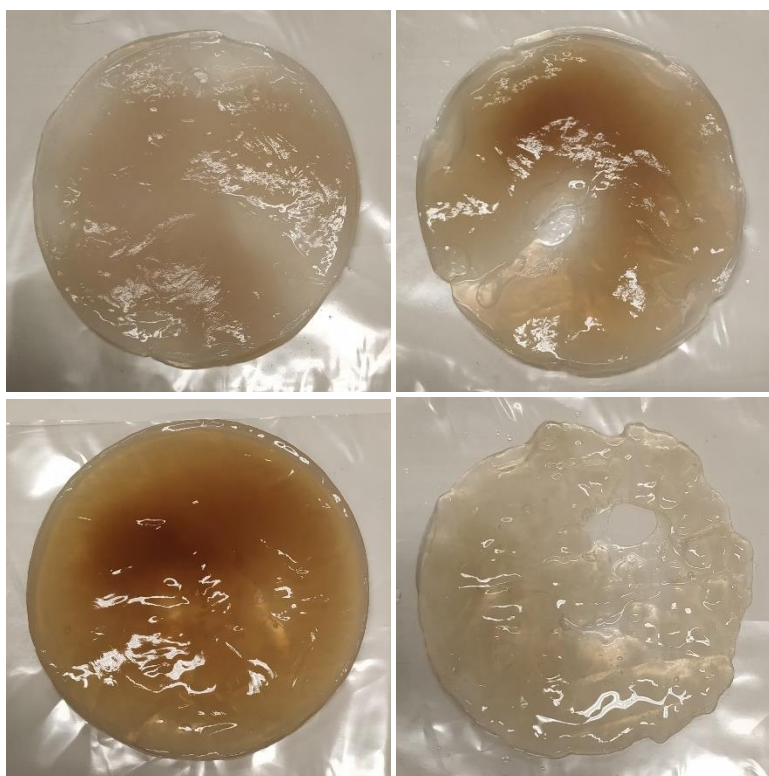
	Caffeine Concentration (mg/L)			
	0 (Control)	300	500	700
BC Yield (g/L)	0.59±0.07 <sup>a</sup>	1.43±0.57 <sup>a</sup>	1.29±0.45 <sup>a</sup>	0.9±0.23 <sup>a</sup>
Tensile Strength (MPa)	29.14±5.06 <sup>a</sup>	32.36±0.86 <sup>a</sup>	14.22±2.21 <sup>b</sup>	31.24±7.94 <sup>a</sup>
Elongation Percentage (%)	1.29±0.31 <sup>a</sup>	2.61±0.33 <sup>c</sup>	1.42±0.38 <sup>a,b</sup>	2.3±0.17 <sup>b,c</sup>
Young's Modulus (MPa)	553.53±0.95 <sup>a</sup>	254.65±2.66 <sup>b</sup>	766.99±26.53 <sup>c</sup>	166.20±6.09 <sup>d</sup>
Water Holding Capacity (%)	216.8±27.9 <sup>a</sup>	183±20.7 <sup>a</sup>	366.1±56.6 <sup>a</sup>	272.4±57.1 <sup>a</sup>
Initial pH	4.75±0.01	4.75±0.02	4.74±0.02	4.76±0.02
Final pH	4.18±0.03	4.24±0.01	4.25±0.01	4.26±0.03

<sup>a,b,c,d</sup> Means in the same row bearing different superscript letters differ significantly ( $P<0.05$ ). Values represent means  $\pm$  standard error of quadruplicate experiments.

### Appendix 4.3. Stress-strain Curve of Samples Produced at Different Caffeine Concentration



**Appendix 5. Result of Experiment 3: Effect of Different Initial pH of Acetate Buffer on the Yield and the Properties of Bacterial Cellulose Biofilms**



**Appendix 5.1. Physical Appearance of BC Produced at Different pH of Acetate Buffer**  
 First row, from left to right: produced in non-buffered and acetate buffered medium at pH 4.0  
 Second row, from left to right: produced in acetate buffered medium at pH 4.75 and 5.5

**Appendix 5.2. Result of Experiment 2**

	pH of 200 mM Acetate Buffer			
	Non Buffered (Control)	4.0	4.75	5.5
BC Yield (g/L)	0.99±0.12 <sup>a</sup>	0.43±0.07 <sup>a</sup>	1.29±0.45 <sup>a</sup>	0.37±0.07 <sup>a</sup>
Tensile Strength (MPa)	23.29±2.98 <sup>a</sup>	28.33±1.84 <sup>a</sup>	14.22±2.21 <sup>b</sup>	32.34±4.73 <sup>a</sup>
Elongation Percentage (%)	3.55±0.19 <sup>a</sup>	0.97±0.28 <sup>b</sup>	1.42±0.38 <sup>b</sup>	1.52±0.64 <sup>b</sup>
Young's Modulus (MPa)	630.91±17.90 <sup>a</sup>	469.44±29.04 <sup>b</sup>	766.99±26.53 <sup>c</sup>	248.34±17.64 <sup>d</sup>
Water Holding Capacity (%)	122.1±18.1 <sup>a</sup>	182.9±19.5 <sup>a</sup>	366.1±56.6 <sup>b</sup>	209.2±23.2 <sup>a</sup>
Initial pH	4.64±0.01	4.10±0.04	4.74±0.02	5.25±0.01
Final pH	3.44±0.01	3.70±0.01	4.25±0.01	4.63±0.04

<sup>a,b,c,d</sup> Means in the same row bearing different superscript letters differ significantly ( $P < 0.05$ ). Values represent means  $\pm$  standard error of quadruplicate experiments.

### Appendix 5.3. Stress-strain Curve of Samples Produced at Different pH of Acetate Buffer

