

ALGINATE-OIL PALM-DERIVED NANOCELLULOSE-MONTMORILLONITE
COMPOSITE FOR LIPASE IMMOBILIZATION AND ITS APPLICATION IN
ETHYL LEVULINATE PRODUCTION

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ETHYL LEVULINATE PRODUCTION

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ABSTRACT

The extension of large-scale oil palm plantations to meet global demands has led to a new environmental challenge. The issue rose from the passive dumping and the open burning of unwanted agriculture biomass i.e. matured oil palm fronds leaves (OPFL). Therefore, a future development toward further technological utilization of OPFL warrants attention of the scientific society. This study capitalized on developing a ternary support that combines nanocellulose (NC) derived from OPFL and montmorillonite (MMT) as reinforcing agents into alginate (ALG) polymer for covalent immobilization of *Candida rugosa* lipase (CRL). Successive treatments of OPFL with bleach, alkali, and acid hydrolysis produced NC in the form of whitish powder. Analysis of NC by attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), thermal gravimetric analysis (TGA), X-ray diffraction (XRD), field emission scanning electron microscopy-energy dispersive X-ray (FESEM-EDX), transmission electron microscopy (TEM) and atomic force microscopy (AFM) confirmed the successful extraction of NC from OPFL. Surface area of NC obtained ($5.11 \text{ m}^2/\text{g}$) was corresponded to a crystallinity index of 45.0%. Meanwhile, the surface showed needle-like structures with diameters of 10–30 nm. Covalent immobilization of CRL onto the ALG/NC/MMT *via* epichlorohydrin attained a maximum enzyme loading and specific activity of 5.80 mg/g and $31.90 \pm 0.80 \text{ U/mg}$, respectively. Data on surface topography and morphology of CRL-ALG/NC/MMT using the same analyses as mentioned above showed that CRL was present on the surface of the ALG/NC/MMT support. The suitability of protocol to immobilize CRL onto the ALG/NC/MMT supports was assessed for factors namely immobilization time, temperature, pH buffer and protein loading, to yield the highest conversion of ethyl levulinate (EL) within 2 h of reaction. Optimal conditions that gave the highest yield of EL (92.89%) using Taguchi design were 7.00 mg/mL protein loading, incubated for 7 h at 35°C and buffer of pH 5, with factors of immobilization time and protein loading displayed the most prominent effect on the process. CRL-ALG/NC/MMT showed an extended operational stability, attaining approximately 50% of its initial activity after nine consecutive esterification cycles. Analyses on purified EL confirmed that the ester was successfully synthesized. Based on the results, it can be concluded that the newly developed ALG/NC/MMT can potentially be employed as a support for lipase immobilization.

ABSTRAK

Peluasan ladang kelapa sawit berskala besar untuk memenuhi tuntutan global telah membawa kepada cabaran alam sekitar yang baharu. Isu ini berpunca daripada pembuangan secara pasif dan pembakaran terbuka biojisim pertanian yang tidak diingini contohnya daun pelepah kelapa sawit yang matang (OPFL). Oleh itu, pembangunan pada masa hadapan ke arah melanjutkan penggunaan teknologi OPFL wajar menarik perhatian masyarakat saintifik. Kajian ini mencuba untuk membangunkan penyokong ternari yang menggabungkan nanoselulosa (NC) daripada OPFL dan montmorillonit (MMT) sebagai agen pengukuh di dalam polimer alginat (ALG) bagi pemegungan kovalen lipase *Candida rugosa* (CRL). Rawatan berturutan OPFL dengan peluntur, alkali, dan hidrolisis berasid telah menghasilkan NC dalam bentuk serbuk putih. Analisis NC oleh spektroskopi inframerah transformasi Fourier pantulan total dilemahkan (ATR-FTIR), analisis termogravimetri (TGA), pembelauan sinar-X (XRD), mikroskopi elektron pengimbas pemancaran medan-serakan tenaga sinar-X (FESEM-EDX), mikroskopi elektron penghantaran (TEM) dan mikroskopi daya atom (AFM) mengesahkan bahawa NC telah berjaya diekstrak daripada OPFL. Luas permukaan NC yang diperoleh ($5.11 \text{ m}^2/\text{g}$) sepadan dengan indeks penghabluran 45.0%. Sementara itu, permukaan menunjukkan struktur seperti jarum berdiameter 10–30 nm. Pemegungan kovalen CRL ke atas ALG/NC/MMT melalui epiklorohidrin telah masing-masing mencapai muatan enzim maksimum dan aktiviti spesifik 5.80 mg/g dan $31.90 \pm 0.80 \text{ U/mg}$. Data topografi permukaan dan morfologi yang diperoleh menggunakan analisis yang sama sebagaimana dinyatakan di atas menunjukkan kewujudan CRL di atas permukaan penyokong ALG/NC/MMT. Kesesuaian protokol untuk memegunkan CRL ke atas penyokong ALG/NC/MMT telah dinilai bagi faktor-faktor iaitu masa pemegungan, suhu, pH penimbal dan muatan protein, untuk menghasilkan penukaran tertinggi etil levulinate (EL) dalam tindakbalas selama 2 jam. Keadaan optimum yang memberikan hasil EL tertinggi (92.89%) menggunakan reka bentuk Taguchi ialah muatan protein 7.00 mg/mL, diinkubasi selama 7 jam pada suhu 35°C dan penimbal pH 5, dengan faktor masa pemegungan dan muatan protein memaparkan kesan yang paling menonjol di dalam proses tersebut. CRL-ALG/NC/MMT menunjukkan kestabilan operasi yang berpanjangan, mencapai kira-kira 50 % daripada aktiviti awal selepas sembilan kitaran pengesteran berturut-turut. Analisis terhadap EL tulen mengesahkan bahawa ester telah berjaya disintesis. Berdasarkan hasil kajian ini, boleh disimpulkan bahawa penyokong ALG/NC/MMT yang baru dihasilkan berpotensi untuk digunakan sebagai bahan penyokong bagi memegunkan lipase.

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LIST OF ABBREVIATIONS

AFM	-	Atomic Force Microscopy
ALG	-	Alginate
ANOVA	-	Analysis of Variance
BSA	-	Bovine Serum Albumin
CNC	-	Cellulose nanocrystal
CNF	-	Cellulose nanofiber
CRL	-	<i>Candida rugosa</i> lipase
EDX	-	Energy Dispersive X-ray
EL	-	Ethyl Levulinate
FESEM	-	Field Emission Scanning Electron Microscopy
FTIR	-	Fourier Transform Infrared Spectroscopy
GC–MS	-	Gas Chromatography–Mass Spectrometry
LA	-	Levulinic Acid
MCF	-	Microcrystalline cellulose
MMT	-	Montmorillonite
NC	-	Nanocellulose
NMR	-	Nuclear Magnetic Resonance
OPFL	-	Oil palm frond leaves
TEM	-	Transmission Electron Microscopy
TEMPO	-	2,2,6,6-Tetramethylpiperidine-1-oxyl radical
TGA	-	Thermal Gravimetric Analysis
UV-vis	-	Ultraviolet-visible
XRD	-	X-ray Diffraction

LIST OF SYMBOLS

°C	-	Degree celcius
g	-	Gram
h	-	Hour
L	-	Liter
mg	-	Milligram
mL	-	Milliliter
M	-	Molar
nm	-	Nanometer
rpm	-	Rotation per minutes
v/v	-	Volume per volume
w/v	-	Weight per volume
w/w	-	Weight per weight
μm	-	Micrometer
%	-	Percentage
U	-	Units
I _c	-	Crystallinity index

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

The increase palm oil demand has resulted in new environmental challenges, mainly associated with the dumping of large quantities of agricultural biomass. In turn, some farmers resort to the rapid yet un-ecofriendly method of ‘slash and burn’ to rid-off agricultural biomass and prepare the land for the next planting season (Saliluddin, 2015). This has often resulted in major regional air pollution (Jain *et al.*, 2014) and elevated health problems associated with poor air quality in countries such as Indonesia and Malaysia as well as other neighbouring countries (Li, 2015; Mahat, 2012). Therefore, this study was focused on finding further applications for the discarded oil palm leaves. The idea in hand is to use the biomass as a source of nanomaterial while provisioning another avenue to fully utilize the biomass for commercial application of oil palm biomass.

The oil palm tree consists largely of three main biopolymers; cellulose (~30–50% wt), hemicelluloses (~19–45% wt), and lignin (~15–35% wt) (Mood *et al.*, 2013). Oil palm frond leaves (OPFL) may serve as a potential source of renewable cellulose to produce functionalized nanocellulose (Mohaiyiddin *et al.*, 2016). Acquiring this bionanomaterial from OPFL seems feasible as the leaves are constantly produced but discarded by plantations. Nanocellulose (NC) is typically valued as a reinforcing filler in other biomaterials such as chitosan, agar and alginate (Elias *et al.*, 2017; Deepa *et al.*, 2016; Shankar and Rhim, 2016). In this study, following extraction and purification of cellulose, the NC is modified and applied as a nano-filler for alginate (ALG/NC), consequently applied as a support material for immobilizing a lipase. Cellulose is among the excellent biomaterials for lipase immobilization, in view of its biodegradability, biocompatibility, good mechanical strength, non-toxicity (Elias *et al.*, 2017) as well as environmental friendly (Kengkhetkit and Amornsakchai, 2012).

This study also proposes to incorporate a second nano-filler i.e. nanoclay from the smectite group into the hybrid composite, by which improvement in the composite properties rely on the benefits of its minute size and useful intercalation property. That said, montmorillonite (MMT) which is the member of smectite groups was selected in this study as one of the components in the composite. It is a 2:1 type phyllosilicate having a crystalline structure with an octahedral layer of aluminium hydroxide flanked by two tetrahedral layers of silica, which explains its commonplace application in various nanocomposite systems (Dean *et al.*, 2007). The excellent reinforcement property of MMT stems from its distinctive high surface area (220–270 m²/g) and platelet thickness of 1 nm (Uyama *et al.*, 2003). Moreover, the natural mineral is easily available, environmental-friendly and economically-viable (Saba *et al.*, 2014). Thus, the study anticipates that the hybrid composite can offer higher enzyme loading of the immobilized lipase and improve the biocatalytic efficiency.

The bulk polymer employed in this study as an enzyme support is alginate (ALG) in the form of sodium alginate. It is a popular biopolymer for such application as it is the only polysaccharide that naturally contains carboxyl groups in each constituent residue (Oladoja *et al.*, 2017). This unique composition explains the various applications of the biopolymer as a functional material (Din, 2016; Rhim, 2004). Being a natural polysaccharide, its inherent properties of being non-toxic, biocompatible and biodegradable, are therefore, expected. However, pure ALG inherently has poor mechanical and gas barrier properties, as well as weak water resistance which limit its application; commonplace issues often associated with biopolymers (Abdollahi *et al.*, 2013). Hence, the blending of ALG with other nanomaterials would be advantageous to enhance the structural integrity and rigidity of the biopolymer. Correspondingly, the incorporation of OPFL-derived NC and MMT into ALG (ALG/NC/MMT) may give rise to a ternary composite with possible interesting enhanced features for enzyme immobilization.

The present study immobilized biocatalyst, *Candida rugosa* lipase (CRL), onto the ALG/NC/MMT support, as the lipase has been well documented such purpose (Elias *et al.*, 2017; Ng and Yang, 2016; Mohamad *et al.*, 2015a). However, free CRL is highly susceptible to premature deactivation under harsh industrial manufacturing

conditions (extreme temperature and pH) and demonstrates low activity in organic solvents (Marzuki *et al.*, 2015a). Hence, the immobilization of CRL onto ALG/NC/MMT would be a feasible solution to activate the enzyme and increase its operational stability.

In this study, the developed CRL-ALG/NC/MMT was used to synthesize an ester, ethyl levulinate (EL), a component of natural aroma of apple commonly used as flavor components in food and pharmaceutical products. The current conventional esterification route to produce EL has a drawback that associated with the use of a high manufacturing temperature (Peng *et al.*, 2011). While it allows rapid and mass production of the ester, the method is energy intensive, incurring large running costs and is environmentally unsound. The reaction carried out at elevated temperatures (150–200°C) also counterproductively degrade the formed ester, alongside a laborious downstream homogenous acid catalyst recovery process. Large quantities of unwanted wastes i.e. salts are generated before pure EL can be obtained (Petersson *et al.*, 2005). The catalyst replacement with CRL-ALG/NC/MMT to catalyze the synthesis of EL may appear useful. The ambient reaction conditions for which CRL catalyzes can prevent the degradation of the produced ester while offering potential cost savings (Borrelli and Trono, 2015; Paroul *et al.*, 2010). Thus, this research work was aimed at preparing a novel biocatalyst using ALG/NC/MMT as highly functional support matrix that would activate CRL activity to catalyze the high yield synthesis of EL.

1.2 Statement of Problem

In view of the underutilization of the oil palm biomass in Malaysia, concerted efforts to find new uses for the biomass merit attention of the scientific community. Utilization of a renewable source of NC acquired from the discarded OPFL in combination with MMT to form a binary nano-filler in the ALG matrix, appears to agree well with the ‘Zero Waste’ initiative. This initiative is one of the three-prong initiatives outlined in the 11th Malaysian Plan, focusing on the development of “Zero wastes” technology. This plan was founded on the need to reduce oil palm biomass and to maximize its commercial potential (Rahman *et al.*, 2013). The approach

champion's the zero-waste technology through effective judicial management of OPFL left over from the post-harvest or post-milling process. Herein, the unwanted OPFL would be converted into value-added commodity i.e. NC.

For this work, versatile CRL is the biocatalyst of choice and its application to catalyze such esterification reactions have been well-documented. The activity of the CRL will be improved by covalent immobilization on a ternary ALG/NC/MMT support. It is worth noting that investigations into the biotechnological feasibility of ternary ALG/NC/MMT biocomposite as support for improving activity of CRL remains unreported, hence an avenue that must be explored and substantiated. It is hypothesized the ALG/NC/MMT ternary support can improve activity and robustness of CRL to catalyze the production of EL. This may be possible judging from the multitude of functional groups present on the support, as well as the stabilizing multi-point crosslinking of the support to the CRL. The additional internal cross-linking of MMT and NC with ALG would prospectively reinforce the ALG and stabilize the support for CRL attachment. The surface area for CRL attachment to the interior of the biopolymer may also be improved while boosting the operational stability of immobilized CRL to withstand prolonged rigorous mechanical forces.

1.3 Research Objectives

This study aims to achieve following three objectives:

1. To prepare and characterize NC extracted from OPFL.
2. To covalently immobilized CRL onto ALG/NC/MMT and characterize the morphology of ALG/NC/MMT ternary support and CRL-ALG/NC/MMT biocatalyst.
3. To optimize the protocol for immobilizing CRL onto ALG/NC/MMT ternary blend using Taguchi approach for a maximum synthesis yield of EL.

1.4 Scope of Study

The scopes of this project involve the preparation of OPFL as the source of cellulosic materials. The leaves were collected from an oil palm plantation surrounding Universiti Teknologi Malaysia (UTM), Johor Bahru. The OPFL are sorted, cleaned, cut, dried and ground prior to pretreatment by chemicals to obtain the cellulose. The cellulose was further purified using both chlorite bleaching and alkali treatment followed by acid hydrolysis to extract the NC. The produced NC was characterized accordingly using Attenuated Total Reflection Fourier Transform Infrared spectroscopy (ATR-FTIR), Thermal Gravimetric Analysis (TGA), X-ray Diffraction (XRD), Nitrogen Adsorption, Field Emission Scanning Electron Microscopy-Energy Dispersive X-ray (FESEM-EDX), Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM).

The ALG/NC/MMT ternary composite was prepared to develop a stable biodegradable support by crosslinking the binary nano-fillers NC and MMT into the ALG polymer. The activation of ALG/NC/MMT was achieved by treatment with epichlorohydrin, followed by immobilization of CRL onto ALG/NC/MMT to produce the biocatalyst CRL-ALG/NC/MMT. The physicochemical properties of the composite and biocatalyst were characterized using ATR-FTIR, XRD, TGA, FESEM-EDX and AFM.

The following part of the study was the optimization of the immobilization protocol of CRL-ALG/NC/MMT, by which ability the of CRL-ALG/NC/MMT to catalyze the rapid and high yield esterification synthesis of EL was used as the benchmark model reaction. The screening experiment were first performed for parameters immobilization time, temperature, pH buffer and enzyme loading, to identify the logic range for each, to be assessed in the Taguchi optimization design. It is worth mentioning here, a poorly selected parameter would render the consequent model ineffective to identify the best condition for the lipase-assisted esterification synthesis of EL. The operational stability study of the produced CRL-ALG/NC/MMT in terms of reusability, thermal stability and leaching were established. Finally,

confirmation of a positive esterification reaction was checked by ATR-FTIR, Nuclear Magnetic Resonance (NMR) and Gas Chromatography-Mass Spectrometry (GC-MS).

1.5 Significance of Study

A novel protocol for the development ALG/NC/MMT ternary composite for supporting CRL was introduced for the first time, with the subsequent lipase showing good efficacy for rapid and high yield esterification synthesis of EL. Most importantly, the source of the NC obtained here is biodegradable as it is from agricultural biomass, which may pave the way its further utilization. Conversion of OPFL into multifunctional support material would generate a new portfolio for manufacture of sustainable products which in turns, contribute to our economic growth.

The protocol developed to fabricate the support material may be used for immobilization and activation of other types of enzymes. Enzymatic production of EL also supports a sustainable and greener manufacturing route of the ester. Also, it does not incur the use of high energy or liberation of unwanted waste.

REFERENCES

- Abdollahi, M., Alboofetileh, M., Rezaei, M., & Behrooz, R. (2013). Comparing physico-mechanical and thermal properties of alginate nanocomposite films reinforced with organic and/or inorganic nanofillers. *Food Hydrocolloids*, *32*(2), 416–424.
- Abitbol, T., Rivkin, A., Cao, Y., Nevo, Y., Abraham, E., Ben-Shalom, T., & Shoseyov, O. (2016). Nanocellulose, a tiny fiber with huge applications. *Current Opinion in Biotechnology*, *39*, 76–88.
- Abou-Taleb, K. A., & Galal, G. F. (2018). A comparative study between one-factor-at-a-time and minimum runs resolution-IV methods for enhancing the production of polysaccharide by *Stenotrophomonas daejeonensis* and *Pseudomonas geniculata*. *Annals of Agricultural Sciences*, *63*(2), 173–180.
- Abraham, E., Deepa, B., Pothan, L. A., Jacob, M., Thomas, S., Cvelbar, U., & Anandjiwala, R. (2011). Extraction of nanocellulose fibrils from lignocellulosic fibres: A novel approach. *Carbohydrate Polymers*, *86*(4), 1468–1475.
- Abu-Danso, E., Srivastava, V., Sillanpää, M., & Bhatnagar, A. (2017). Pretreatment assisted synthesis and characterization of cellulose nanocrystals and cellulose nanofibers from absorbent cotton. *International Journal of Biological Macromolecules*, *102*, 248–257.
- Adewale, P., Vithanage, L. N., & Christopher, L. (2017). Optimization of enzyme-catalyzed biodiesel production from crude tall oil using Taguchi method. *Energy Conversion and Management*, *154*, 81–91.
- Adnani, A., Basri, M., Chaibakhsh, N., Ahangar, H., Bakar Salleh, A., Rahman, R., & Rahman, M. (2011). Chemometric analysis of lipase-catalyzed synthesis of xylitol esters in a solvent-free system. *Carbohydrate Research*, *346*(4), 472–479.
- Ahmad, R., & Sardar, M. (2015). Enzyme immobilization: An overview on nanoparticles as immobilization matrix. *Biochemistry & Analytical Biochemistry*, *4*, 178–185.
- Ahmmad, B., Agustin, M. B., & Hirose, F. (2014). Starch based biocomposite films reinforced with cellulose nanocrystals from garlic stalks and rice straw. *Abstracts of Papers of the American Chemical Society*, *247*, 1–2.

- Alemdar, A., & Sain, M. (2008). Isolation and characterization of nanofibers from agricultural residues—wheat straw and soy hulls. *Bioresource Technology*, *99*(6), 1664–1671.
- Ali, Z., Tian, L., Zhang, B., Ali, N., Khan, M., & Zhang, Q. (2017). Synthesis of paramagnetic dendritic silica nanomaterials with fibrous pore structure (Fe₃O₄@KCC-1) and their application in immobilization of lipase from *Candida rugosa* with enhanced catalytic activity and stability. *New Journal of Chemistry*, *41*(16), 8222–8231.
- Aljuboori, A. H. R. (2013). Oil palm biomass residue in Malaysia: availability and sustainability. *International Journal of Biomass & Renewables*, *2*(1), 13–18.
- Andualema, B., & Gessesse, A. (2012). Microbial lipases and their industrial applications: review. *Biotechnology*, *11*(3), 100–118.
- Antranikian, G., Bornscheuer, U. T., & Liese, A. (2010). Highlights in biocatalysis. *ChemCatChem*, *2*(8), 879–880.
- Awalludin, M. F., Sulaiman, O., Hashim, R., & Nadhari, W. N. A. W. (2015). An overview of the oil palm industry in Malaysia and its waste utilization through thermochemical conversion, specifically via liquefaction. *Renewable and Sustainable Energy Reviews*, *50*, 1469–1484.
- Bacakova, L., Pajorova, J., Bacakova, M., Skogberg, A., Kallio, P., Kolarova, K., & Svorcik, V. (2019). Versatile application of nanocellulose: from industry to skin tissue engineering and wound healing. *Nanomaterials*, *9*(2), 164.
- Badgajar, K. C., Dhake, K. P., & Bhanage, B. M. (2013). Immobilization of *Candida cylindracea* lipase on poly lactic acid, polyvinyl alcohol and chitosan based ternary blend film: characterization, activity, stability and its application for N-acylation reactions. *Process Biochemistry*, *48*(9), 1335–1347.
- Bai, W., Holbery, J., & Li, K. (2009). A technique for production of nanocrystalline cellulose with a narrow size distribution. *Cellulose*, *16*(3), 455–465.
- Bart, J. C. J., Palmeri, N., & Cavallaro, S. (2010). Transesterification processes for biodiesel production from oils and fats. In: Bart, J. C. J., Palmeri, N., & Cavallaro, S. ed. , *Biodiesel Science and Technology From Soil to Oil*. United States: Woodhead Publishing. 285–321; 2010.
- Bassegoda, A., Cesarini, S., & Diaz, P. (2012). Lipase improvement: goals and strategies. *Computational and Structural Biotechnology Journal*, *2*(3), 1–8.
- Ben Akacha, N., & Gargouri, M. (2015). Microbial and enzymatic technologies used

- for the production of natural aroma compounds: Synthesis, recovery modeling, and bioprocesses. *Food and Bioproducts Processing*, 94, 675–706.
- Bisswanger, H. (2014). Enzyme assays. *Perspectives in Science*, 1(1), 41–55.
- Bof, M. J., Bordagaray, V. C., Locaso, D. E., & García, M. A. (2015). Chitosan molecular weight effect on starch-composite film properties. *Food Hydrocolloids*, 51, 281–294.
- Borrelli, G., & Trono, D. (2015). Recombinant lipases and phospholipases and their use as biocatalysts for industrial applications. *International Journal of Molecular Sciences*, 16(9), 20774–20840.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1–2), 248–254.
- Bragd, P., Besemer, A., & van Bekkum, H. (2002). Selective oxidation of carbohydrates by 4-AcNH-TEMPO/peracid systems. *Carbohydrate Polymers*, 49(4), 397–406.
- Camarero Espinosa, S., Kuhnt, T., Foster, E. J., & Weder, C. (2013). Isolation of thermally stable cellulose nanocrystals by phosphoric acid hydrolysis. *Biomacromolecules*, 14(4), 1223–1230.
- Cao, S.-L., Huang, Y. M., Li, X. H., Xu, P., Wu, H., Li, N., & Zong, M. H. (2016). Preparation and characterization of immobilized lipase from *Pseudomonas cepacia* onto magnetic cellulose nanocrystals. *Scientific Reports*, 6, 1–12.
- Chakraborty, R., & RoyChowdhury, D. (2013). Fish bone derived natural hydroxyapatite-supported copper acid catalyst: Taguchi optimization of semibatch oleic acid esterification. *Chemical Engineering Journal*, 215–216, 491–499.
- Chamoli, S. (2015). A Taguchi approach for optimization of flow and geometrical parameters in a rectangular channel roughened with V down perforated baffles. *Case Studies in Thermal Engineering*, 5, 59–69.
- Chang, S. W., Lee, G. C., & Shaw, J. F. (2006). Codon optimization of *Candida rugosa* LIP1 gene for improving expression in *pichia pastoris* and biochemical characterization of the purified recombinant LIP1 lipase. *Journal of Agricultural and Food Chemistry*, 54(3), 815–822.
- Chapman, J., Ismail, A., & Dinu, C. (2018). Industrial applications of enzymes: Recent advances, techniques, and outlooks. *Catalysts*, 8(6), 238.

- Cherian, B. M., Leão, A. L., De Souza, S. F., Thomas, S., Pothan, L. A., & Kottaisamy, M. (2010). Isolation of nanocellulose from pineapple leaf fibres by steam explosion. *Carbohydrate Polymers*, *81*(3), 720–725.
- Chieng, B., Lee, S., Ibrahim, N., Then, Y., & Loo, Y. (2017). Isolation and characterization of cellulose nanocrystals from oil palm mesocarp fiber. *Polymers*, *9*(8), 355.
- Chowdhury, A., Chakraborty, R., Mitra, D., & Biswas, D. (2014). Optimization of the production parameters of octyl ester biolubricant using Taguchi's design method and physico-chemical characterization of the product. *Industrial Crops and Products*, *52*, 783–789.
- Chunilall, V., Bush, T., & Larsson, P. T. Supra-molecular structure and chemical reactivity of cellulose I studied using CP/MAS ¹³C-NMR. In: Ven, T. V. D., & Godbout, L. ed. *Cellulose-Fundamental Aspects*. Croatia: Intech. 69–76. 2013.
- Cihangir, N., & Sarikaya, E. (2004). Investigation of lipase production by a new isolate of *Aspergillus* sp. *World Journal of Microbiology and Biotechnology*, *20*(2), 193–197.
- Datta, S., Christena, R., & Rani Sriramulu Rajaram, Y. (2012). Enzyme immobilization: An overview on techniques and support materials. *3 Biotech*, *3*(1), 1–9.
- Davoudpour, Y., Hossain, S., Khalil, H. P. S. A., Haafiz, M. K. M., Ishak, Z. A. M., Hassan, A., & Sarker, Z. I. (2015). Optimization of high pressure homogenization parameters for the isolation of cellulosic nanofibers using response surface methodology. *Industrial Crops and Products*, *74*, 381–387.
- Dean, K., Yu, L., & Wu, D. Y. (2007). Preparation and characterization of melt-extruded thermoplastic starch/clay nanocomposites. *Composites Science and Technology*, *67*(3–4), 413–421.
- Deepa, B., Abraham, E., Cordeiro, N., Mozetic, M., Mathew, A. P., Oksman, K., & Pothan, L. A. (2015). Utilization of various lignocellulosic biomass for the production of nanocellulose: A comparative study. *Cellulose*, *22*(2), 1075–1090.
- Deepa, B., Abraham, E., Pothan, L., Cordeiro, N., Faria, M., & Thomas, S. (2016). Biodegradable nanocomposite films based on sodium alginate and cellulose nanofibrils. *Materials*, *9*(1), 50.
- Dhawane, S. H., Bora, A. P., Kumar, T., & Halder, G. (2017). Parametric optimization of biodiesel synthesis from rubber seed oil using iron doped carbon catalyst by

- Taguchi approach. *Renewable Energy*, 105, 616–624.
- Dhawane, S. H., Kumar, T., & Halder, G. (2016). Biodiesel synthesis from *Hevea brasiliensis* oil employing carbon supported heterogeneous catalyst: optimization by Taguchi method. *Renewable Energy*, 89, 506–514.
- Din, H. U. *Programmable Biopolymers for theranostic applications*. Ph.D. Thesis. Norwegian University of Science and Technology; 2016.
- Domínguez de María, P., Sánchez-Montero, J. M., Sinisterra, J. V., & Alcántara, A. R. (2006). Understanding *Candida rugosa* lipases: an overview. *Biotechnology Advances*, 24(2), 180–196.
- Dufresne, A. (2013). Nanocellulose: a new ageless bionanomaterial. *Materials Today*, 16(6), 220–227.
- Dufresne, A. *Nanocellulose: from nature to high performance tailored materials*. 2nd Edition. Berlin: Walter de Gruyter GmbH & Co KG. 2017.
- Dungani, R., Owolabi, A. F., Saurabh, C. K., Khalil, H. P. S. A., Tahir, P. M., Hazwan, C., & Aditiawati, P. (2017). Preparation and fundamental characterization of cellulose nanocrystal from oil palm fronds biomass. *Journal of Polymers and The Environment*, 25(3), 692–700.
- Elias, N., Chandren, S., Attan, N., Mahat, N. A., Razak, F. I. A., Jamalis, J., & Wahab, R. A. (2017). Structure and properties of oil palm-based nanocellulose reinforced chitosan nanocomposite for efficient synthesis of butyl butyrate. *Carbohydrate Polymers*, 176, 281–292.
- Elias, N., Chandren, S., Razak, F. I. A., Jamalis, J., Widodo, N., & Wahab, R. A. (2018). Characterization, optimization and stability studies on *Candida rugosa* lipase supported on nanocellulose reinforced chitosan prepared from oil palm biomass. *International Journal of Biological Macromolecules*, 114, 306–316.
- Elias, N., Wahab, R., Chandren, S., Razak, F., & Jamalis, J. (2019). Effect of operative variables and kinetic study of butyl butyrate synthesis by *Candida rugosa* lipase activated by chitosan-reinforced nanocellulose derived from raw oil palm leaves. *Enzyme and Microbial Technology*, 130, 1–11.
- Elnashar, M. (2010). Immobilized molecules using biomaterials and nanobiotechnology. *Journal of Biomaterials and Nanobiotechnology*, 1, 61–77.
- Essa, H., Magner, E., Cooney, J., & Hodnett, B. K. (2007). Influence of pH and ionic strength on the adsorption, leaching and activity of myoglobin immobilized onto ordered mesoporous silicates. *Journal of Molecular Catalysis B-Enzymatic*, 49,

- 61–68.
- Fadılođlu, S., & Söylemez, Z. (1998). Olive oil hydrolysis by celite-immobilized *Candida rugosa* lipase. *Journal of Agricultural and Food Chemistry*, *46*(9), 3411–3414.
- Fard Masoumi, H. R., Kassim, A., Basri, M., & Kuang Abdullah, D. (2011). Determining optimum conditions for lipase-catalyzed synthesis of triethanolamine (TEA)-based esterquat cationic surfactant by a Taguchi robust design method. *Molecules*, *16*(6), 4672–4680.
- Fehér, A. (2017). Combined approaches to xylose production from corn stover by dilute acid hydrolysis. *Chemical and Biochemical Engineering Quarterly*, *31*(1), 77–87.
- Feltes, M., de Oliveira, D., Ninow, J., & Oliveira, J. An overview of enzyme-catalyzed reactions and alternative feedstock for biodiesel production. In: Manzanera, M. *Alternative Fuel*. United States: InTech Open. 175–188. 2011.
- Filson, P. B., & Dawson-Andoh, B. E. (2009). Sono-chemical preparation of cellulose nanocrystals from lignocellulose derived materials. *Bioresource Technology*, *100*(7), 2259–2264.
- Freitas, L., Paula, A. V, dos Santos, J. C., Zanin, G. M., & de Castro, H. F. (2010). Enzymatic synthesis of monoglycerides by esterification reaction using *Penicillium camembertii* lipase immobilized on epoxy SiO₂-PVA composite. *Journal of Molecular Catalysis B: Enzymatic*, *65*(1), 87–90.
- Frone, A. N., Panaitescu, D. M., & Donescu, D. (2011). Some aspects concerning the isolation of cellulose micro- and nano-fibers. *UPB Buletin Stiintific, Series B: Chemistry and Materials Science*, *73*(2), 133–152.
- Garces, I. T., Aslanzadeh, S., Boluk, Y., & Ayranci, C. (2018). Cellulose nanocrystals (CNC) reinforced shape memory polyurethane ribbons for future biomedical applications and design. *Journal of Thermoplastic Composite Materials*, *31*, 1–16.
- George, J., & Sabapathi, S. N. (2015). Cellulose nanocrystals: synthesis, functional properties, and applications. *Nanotechnology, Science and Applications*, *8*, 45–54.
- Goh, C. S., Tan, K. T., Lee, K. T., & Bhatia, S. (2010). Bio-ethanol from lignocellulose: status, perspectives and challenges in Malaysia. *Bioresource Technology*, *101*(13), 4834–4841.

- Golbaha, N., Ramli, Z., & Endud, S. (2016). Immobilization of lipase onto mesoporous silica KIT-6 and montmorillonite K10 for enzymatic hydrolysis of tributyrin. *Malaysian Journal of Fundamental and Applied Sciences*, *12*(1), 39–46.
- Gopinath, S., & Sugunan, S. (2004). Leaching studies over immobilized α -amylase. Importance of the nature of enzyme attachment. *Reaction Kinetics and Catalysis Letters*, *83*, 79–83.
- Guisan, J. *Immobilization of Enzymes and Cells*. 2nd Edition. United States: Humana Press. 2006.
- Guo, J., & Catchmark, J. M. (2012). Surface area and porosity of acid hydrolyzed cellulose nanowhiskers and cellulose produced by *Gluconacetobacter xylinus*. *Carbohydrate Polymers*, *87*(2), 1026–1037.
- Guzik, U., Hupert-Kocurek, K., & Wojcieszynska, D. (2014). Immobilization as a strategy for improving enzyme properties-application to oxidoreductases. *Molecules*, *19*(7), 8995–9018.
- Habibi, Y., Lucia, L. A., & Rojas, O. J. (2010). Cellulose nanocrystals: chemistry, self-assembly, and applications. *Chemical Reviews*, *110*(6), 3479–3500.
- Hagström, A., Tufvesson, L., Nordblad, M., Börjesson, P., Mattiasson, B., & Adlercreutz, P. (2005). Wax esters produced by solvent-free energy-efficient enzymatic synthesis and their applicability as wood coatings. *Green Chemistry*, *7*, 837–843.
- Hari-Krishna, S. (2002). Developments and trends in enzyme catalysis in nonconventional media. *Biotechnology Advances*, *20*(3), 239–267.
- Hasanah, A. N., Muhtadi, A., Elyani, I., & Musfiroh, I. (2015). Epichlorohydrin as crosslinking agent for synthesis of carboxymethyl cellulose sodium (Na-CMC) as pharmaceutical excipient from water hyacinth (*Eichornia crassipes* L.). *International Journal of Chemical Science*, *13*(3), 1227–1237.
- Hassan, S. Z., & Vinjamur, M. (2014). Parametric effects on kinetics of esterification for biodiesel production: a Taguchi approach. *Chemical Engineering Science*, *110*, 94–104.
- Herlet, J., Kornberger, P., Roessler, B., Glanz, J., Schwarz, W., Liebl, W., & Zverlov, V. (2017). A new method to evaluate temperature vs. pH activity profiles for biotechnological relevant enzymes. *Biotechnology for Biofuels*, *10*, 1–12.
- Hong, B., Chen, F., & Xue, G. (2016). Preparation and characterization of cellulose nanocrystals from bamboo pulp. *Cellulose Chemistry & Technology*, *50*(2), 225–

231.

- Hu, J., Tian, D., Renneckar, S., & Saddler, J. N. (2018). Enzyme mediated nanofibrillation of cellulose by the synergistic actions of an endoglucanase, lytic polysaccharide monooxygenase (LPMO) and xylanase. *Scientific Reports*, 8(1), 3195–3202.
- Hua, S., Ma, H., Li, X., Yang, H., & Wang, A. (2010). pH-sensitive sodium alginate/poly (vinyl alcohol) hydrogel beads prepared by combined Ca²⁺ crosslinking and freeze-thawing cycles for controlled release of diclofenac sodium. *International Journal of Biological Macromolecules*, 46(5), 517–523.
- Huang, X. J., Chen, P. C., Huang, F., Ou, Y., Chen, M. R., & Xu, Z. K. (2011). Immobilization of *Candida rugosa* lipase on electrospun cellulose nanofiber membrane. *Journal of Molecular Catalysis B: Enzymatic*, 70(3–4), 95–100.
- Huq, T., Salmieri, S., Khan, A., Khan, R. A., Le Tien, C., Riedl, B., & Kamal, M. R. (2012). Nanocrystalline cellulose (NCC) reinforced alginate based biodegradable nanocomposite film. *Carbohydrate Polymers*, 90(4), 1757–1763.
- Ikeda, A., Takemura, A., & Ono, H. (2000). Preparation of low-molecular weight alginic acid by acid hydrolysis. *Carbohydrate Polymers*, 42(4), 421–425.
- Ioelovich, M. (2012). Optimal conditions for isolation of nanocrystalline cellulose particles. *Journal of Nanoscience and Nanotechnology*, 2(2), 9–13.
- Isah, A. A., Mahat, N. A., Jamalis, J., Attan, N., Zakaria, I. I., Huyop, F., & Wahab, R. A. (2017). Synthesis of geranyl propionate in a solvent-free medium using *Rhizomucor miehei* lipase covalently immobilized on chitosan–graphene oxide beads. *Preparative Biochemistry and Biotechnology*, 47(2), 199–210.
- Isikgor, F. H., & Becer, C. R. (2015). Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers. *Polymer Chemistry*, 6(25), 4497–4559.
- Isogai, T., Saito, T., & Isogai, A. (2011). Wood cellulose nanofibrils prepared by TEMPO electro-mediated oxidation. *Cellulose*, 18(2), 421–431.
- Jain, N., Bhatia, A., & Pathak, H. (2014). Emission of air pollutants from crop residue burning in India. *Aerosol and Air Quality Research*, 14(1), 422–430.
- Jamie, A., Alshami, A. S., Maliabari, Z. O., Ali Ateih, M., & Al Hamouz, O. C. S. (2016). Immobilization and enhanced catalytic activity of lipase on modified MWCNT for oily wastewater treatment. *Environmental Progress & Sustainable Energy*, 35(5), 1441–1449.

- Jia, Y., Wang, X., Huo, M., Zhai, X., Li, F., & Zhong, C. (2017). Preparation and characterization of a novel bacterial cellulose/chitosan bio-hydrogel. *Nanomaterials and Nanotechnology*, 7, 1–8.
- Jiang, W., Liu, S., Zhou, C., Gao, S., Song, Y., Li, W., & Han, G. (2018). Preparation of nanocellulose directly from kenaf bast: the change in particle size. *BioResources*, 13(3), 5598–5607.
- Jönsson, L. J., & Martín, C. (2016). Pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimizing their effects. *Bioresource Technology*, 199, 103–112.
- Joshi, H., Moser, B., Toler, J., F. Smith, W., & Walker, T. (2011). Ethyl levulinate: a potential bio-based diluent for biodiesel which improves cold flow properties. *Biomass & Bioenergy*, 35(7), 3263–3266.
- Kang, X., Sun, P., Kuga, S., Wang, C., Zhao, Y., Wu, M., & Huang, Y. (2017). Thin cellulose nanofiber from corncob cellulose and its performance in transparent nanopaper. *ACS Sustainable Chemistry & Engineering*, 5(3), 2529–2534.
- Kargarzadeh, H., Ahmad, I., Thomas, S., & Dufresne, A. *Handbook of nanocellulose and cellulose nanocomposites*. Germany: Wiley–VCH. 2017.
- Kargarzadeh, H., Ishak, bullet, Ahmad, I., Abdullah, I., Dufresne, A., & Sheltami, M. (2012). Effects of hydrolysis conditions on the morphology, crystallinity, and thermal stability of cellulose nanocrystals extracted from kenaf bast fibers. In *Cellulose*, 19(3), 855–866.
- Karinkanta, P., Ämmälä, A., Illikainen, M., & Niinimäki, J. (2018). Fine grinding of wood—overview from wood breakage to applications. *Biomass and Bioenergy*, 113, 31–44.
- Kasirga, Y., Oral, A., & Caner, C. (2012). Preparation and characterization of chitosan/montmorillonite-K10 nanocomposites films for food packaging applications. *Polymer Composites*, 33(11), 1874–1882.
- Kengkhetkit, N., & Amornsakchai, T. (2012). Utilisation of pineapple leaf waste for plastic reinforcement: a novel extraction method for short pineapple leaf fiber. *Industrial Crops and Products*, 40, 55–61.
- Kevadiya, B. D., Patel, H. A., Joshi, G. V., Abdi, S. H. R., & Bajaj, H. C. (2010). Montmorillonite-alginate composites as a drug delivery system: intercalation and in vitro release of diclofenac sodium. *Indian Journal of Pharmaceutical Sciences*, 72(6), 732.

- Khalil, H. P. S. A., Davoudpour, Y., Islam, M. N., Mustapha, A., Sudesh, K., Dungani, R., & Jawaid, M. (2014). Production and modification of nanofibrillated cellulose using various mechanical processes: a review. *Carbohydrate Polymers*, *99*, 649–665.
- Khalil, H. P. S. A., Davoudpour, Y., Saurabh, C. K., Hossain, M. S., Adnan, A. S., Dungani, R., & Syakir, M. I. (2016). A review on nanocellulosic fibres as new material for sustainable packaging: process and applications. *Renewable and Sustainable Energy Reviews*, *64*, 823–836.
- Khan, N., Maseet, M., & Basir, S. F. (2017). Enhanced biocatalytic esterification with lipase- immobilized glutaraldehyde crosslinked chitosan. *World Journal of Pharmacy and Pharmaceutical Sciences*, *6*(1), 1268–1282.
- Kiran, K. R., Krishna, S. H., Babu, C. V. S., Karanth, N. G., & Divakar, S. (2000). An esterification method for determination of lipase activity. *Biotechnology Letters*, *22*(19), 1511–1514.
- Kumar, N., & Kumbhat, S. *Essentials in nanoscience and nanotechnology*. United States: John Wiley & Sons Inc. 2016.
- Kumar, U., Sharma, P., Singh, K. L., & Kumar, A. (2018). Extraction and characterization of nanocellulose from saw dust. *Nano Trends-A Journal of Nano Technology and Its Applications*, *19*(3), 43–48.
- Kuperkar, V. V., Lade, V. G., Prakash, A., & Rathod, V. K. (2014). Synthesis of isobutyl propionate using immobilized lipase in a solvent free system: optimization and kinetic studies. *Journal of Molecular Catalysis B: Enzymatic*, *99*, 143–149.
- Kushairi, A., Loh, S. K., Azman, I., Hishamuddin, E., Ong-Abdullah, M., Izuddin, Z., & Parveez, G. K. A. (2018). Oil palm economic performance in Malaysia and R&D progress in 2017. *Journal of Oil Palm Research*, *30*(2), 163–195.
- Kusuktham, B., Prasertgul, J., & Srinun, P. (2014). Morphology and property of calcium silicate encapsulated with alginate beads. *Silicon*, *6*(3), 191–197.
- Lahiji, R. R., Xu, X., Reifenberger, R., Raman, A., Rudie, A., & Moon, R. J. (2010). Atomic force microscopy characterization of cellulose nanocrystals. *Langmuir*, *26*(6), 4480–4488.
- Lai, J. Q., Li, Z., Lü, Y. H., & Yang, Z. (2011). Specific ion effects of ionic liquids on enzyme activity and stability. *Green Chemistry*, *13*(7), 1860–1868.
- Lamaming, J., Hashim, R., Leh, C. P., Sulaiman, O., Sugimoto, T., & Nasir, M. (2015).

- Isolation and characterization of cellulose nanocrystals from parenchyma and vascular bundle of oil palm trunk (*Elaeis guineensis*). *Carbohydrate Polymers*, *134*, 534–540.
- Lang, X., Zhu, L., Gao, Y., & Wheeldon, I. (2017). Enhancing enzyme activity and immobilization in nanostructured inorganic–enzyme complexes. *Langmuir*, *33*(36), 9073–9080.
- Lani, N. S., Ngadi, N., Johari, A., & Jusoh, M. (2014). Isolation, Characterization, and Application of Nanocellulose from Oil Palm Empty Fruit Bunch Fiber as Nanocomposites. *Journal of Nanomaterials*, *2014*, 1–9.
- Le Troedec, M., Sedan, D., Peyratout, C., Bonnet, J. P., Smith, A., Guinebretiere, R., & Krausz, P. (2008). Influence of various chemical treatments on the composition and structure of hemp fibres. *Composites Part A: Applied Science and Manufacturing*, *39*(3), 514–522.
- Lee, A., Chaibakhsh, N., Rahman, M. B. A., Basri, M., & Tejo, B. A. (2010). Optimized enzymatic synthesis of levulinate ester in solvent-free system. *Industrial Crops and Products*, *32*(3), 246–251.
- Lee, K. Y., Aitomäki, Y., Berglund, L. A., Oksman, K., & Bismarck, A. (2014a). On the use of nanocellulose as reinforcement in polymer matrix composites. *Composites Science and Technology*, *105*, 15–27.
- Lee, K. Y., Rowley, J. A., Eiselt, P., Moy, E. M., Bouhadir, K. H., & Mooney, D. J. (2000). Controlling mechanical and swelling properties of alginate hydrogels independently by cross-linker type and cross-linking density. *Macromolecules*, *33*(11), 4291–4294.
- Lee, H. V, Hamid, S. B. A., & Zain, S. K. (2014b). Conversion of lignocellulosic biomass to nanocellulose: structure and chemical process. *The Scientific World Journal*, *2014*, 1–20.
- Leitner, J., Hinterstoisser, B., Wastyn, M., Keckes, J., & Gindl, W. (2007). Sugar beet cellulose nanofibril-reinforced composites. *Cellulose*, *14*(5), 419–425.
- Lenfant, N., Hotelier, T., Bourne, Y., Marchot, P., & Chatonnet, A. (2013). Proteins with an alpha/beta hydrolase fold: relationships between subfamilies in an ever-growing superfamily. *Chemico-Biological Interactions*, *203*(1), 266–268.
- Leszczyńska, A., Njuguna, J., Pielichowski, K., & Banerjee, J. R. (2007). Polymer/montmorillonite nanocomposites with improved thermal properties: part i. factors influencing thermal stability and mechanisms of thermal stability

- improvement. *Thermochimica Acta*, 453(2), 75–96.
- Levanič, J., Poljanšek, I., & Oven, P. (2018). Chlorine-free method for the oxidation of residual aldehydes on TEMPO-oxidized cellulose. *BioResources*, 13(4), 7969–7982.
- Li, J., Wei, X., Wang, Q., Chen, J., Chang, G., Kong, L., & Liu, Y. (2012). Homogeneous isolation of nanocellulose from sugarcane bagasse by high pressure homogenization. *Carbohydrate Polymers*, 90(4), 1609–1613.
- Li, T. M. *Social impacts of oil palm in Indonesia: A gendered perspective from West Kalimantan*. Bogor, Indonesia: Center for International Forestry Research (CIFOR). 2015.
- Lin, N., Huang, J., Chang, P. R., Feng, L., & Yu, J. (2011). Effect of polysaccharide nanocrystals on structure, properties, and drug release kinetics of alginate-based microspheres. *Colloids and Surfaces B: Biointerfaces*, 85(2), 270–279.
- Lin, N., Huang, J., & Dufresne, A. (2012). Preparation, properties and applications of polysaccharide nanocrystals in advanced functional nanomaterials: a review. *Nanoscale*, 4(11), 3274–3294.
- Liu, H., Liu, D., Yao, F., & Wu, Q. (2010). Fabrication and properties of transparent polymethylmethacrylate/cellulose nanocrystals composites. *Bioresource Technology*, 101(14), 5685–5692.
- Liu, W., Dong, Y., Liu, D., Bai, Y., & Lu, X. (2018). Polylactic acid (PLA)/cellulose nanowhiskers (CNWs) composite nanofibers: microstructural and properties analysis. *Journal of Composites Science*, 2(1), 1–14.
- Liu, X., Chen, X., Li, Y., Cui, Y., Zhu, H., & Zhu, W. (2012). Preparation of superparamagnetic sodium alginate nanoparticles for covalent immobilization of *Candida rugosa* lipase. *Journal of Nanoparticle Research*, 14(3), 763.
- Lu, P., & Hsieh, Y. L. (2010). Preparation and properties of cellulose nanocrystals: rods, spheres, and network. *Carbohydrate Polymers*, 82(2), 329–336.
- Luzi, F., Fortunati, E., Puglia, D., Lavorgna, M., Santulli, C., Kenny, J. M., & Torre, L. (2014). Optimized extraction of cellulose nanocrystals from pristine and carded hemp fibres. *Industrial Crops and Products*, 56, 175–186.
- Ma, H., Burger, C., Hsiao, B. S., & Chu, B. (2014). Fabrication and characterization of cellulose nanofiber based thin-film nanofibrous composite membranes. *Journal of Membrane Science*, 454, 272–282.
- Mahardika, M., Abral, H., Kasim, A., Arief, S., & Asrofi, M. (2018). Production of

- nanocellulose from pineapple leaf fibers via high-shear homogenization and ultrasonication. *Fibers*, 6(2), 28.
- Mahat, S. B. A. The palm oil industry from the perspective of sustainable development: a case study of Malaysian palm oil industry. Master Thesis. Graduate School of Asia Pacific Studies, Ritsumeikan Asia Pacific University of Japan; 2012.
- Maheria, K., Kozinski, J., & Dalai, A. (2013). Esterification of levulinic acid to n-butyl levulinate over various acidic zeolites. *Catalysis Letters*, 143(11), 1220–1225.
- Manan, F. M. A., Attan, N., Zakaria, Z., Keyon, A. S. A., & Wahab, R. A. (2018). Enzymatic esterification of eugenol and benzoic acid by a novel chitosan-chitin nanowhiskers supported Rhizomucor miehei lipase: process optimization and kinetic assessments. *Enzyme and Microbial Technology*, 108, 42–52.
- Manan, F. M. A., Attan, N., Zakaria, Z., Mahat, N. A., & Wahab, R. A. (2018). Insight into the Rhizomucor miehei lipase supported on chitosan-chitin nanowhiskers assisted esterification of eugenol to eugenyl benzoate. *Journal of Biotechnology*, 280, 19–30.
- Manan, F. M. A., Rahman, I. N. A., Marzuki, N. H. C., Mahat, N. A., Huyop, F., & Wahab, R. A. (2016). Statistical modelling of eugenol benzoate synthesis using Rhizomucor miehei lipase reinforced nanobioconjugates. *Process Biochemistry*, 51(2), 249–262.
- Mariano, M., Cercená, R., & Soldi, V. (2016). Thermal characterization of cellulose nanocrystals isolated from sisal fibers using acid hydrolysis. *Industrial Crops and Products*, 94, 454–462.
- Mariño, M., Lopes da Silva, L., Durán, N., & Tasic, L. (2015). Enhanced materials from nature: nanocellulose from citrus waste. *Molecules*, 20(4), 5908–5923.
- Mary, C. S., & Swamiappan, S. (2016). Sodium alginate with PEG/PEO blends as a floating drug delivery carrier—in vitro evaluation. *Advanced Pharmaceutical Bulletin*, 6(3), 435.
- Marzuki, N. H. C., Huyop, F., Aboul-Enein, H. Y., Mahat, N. A., & Wahab, R. A. (2015a). Modelling and optimization of Candida rugosa nanobioconjugates catalysed synthesis of methyl oleate by response surface methodology. *Biotechnology & Biotechnological Equipment*, 29(6), 1113–1127.
- Marzuki, N. H. C., Mahat, N. A., Huyop, F., Aboul-Enein, H. Y., & Wahab, R. A. (2015b). Sustainable production of the emulsifier methyl oleate by Candida

- rugosa lipase nanoconjugates. *Food and Bioproducts Processing*, 96, 211–220.
- Mathew, A., Chaney, K., Crook, M., & Claire Humphries, A. (2011). Alkaline pre-treatment of oilseed rape straw for bioethanol production: evaluation of glucose yield and pre-treatment energy consumption. *Bioresource technology*, 102(11), 6547–6553.
- Mazlan, S. Z., & Hanifah, S. A. (2017). Effects of temperature and pH on immobilized laccase activity in conjugated methacrylate-acrylate microspheres. *International Journal of Polymer Science*, 2017, 1–8.
- Mehrotra, P. (2016). Biosensors and their applications - a review. *Journal of Oral Biology and Craniofacial Research*, 6(2), 153–159.
- Mendes, A. A., de Castro, H. F., Andrade, G. S. S., Tardioli, P. W., & de LC Giordano, R. (2013). Preparation and application of epoxy–chitosan/alginate support in the immobilization of microbial lipases by covalent attachment. *Reactive and Functional Polymers*, 73(1), 160–167.
- Mendes, A. A., Giordano, R. C., de LC Giordano, R., & de Castro, H. F. (2011). Immobilization and stabilization of microbial lipases by multipoint covalent attachment on aldehyde-resin affinity: application of the biocatalysts in biodiesel synthesis. *Journal of Molecular Catalysis B: Enzymatic*, 68(1), 109–115.
- Mihailović, M., Stojanović, M., Banjanac, K., Carević, M., Prlainović, N., Milosavić, N., & Bezbradica, D. (2014). Immobilization of lipase on epoxy-activated Purolite® A109 and its post-immobilization stabilization. *Process Biochemistry*, 49(4), 637–646.
- Mohaiyiddin, M. S., Lin, O. H., Owi, W. T., Chan, C. H., Chia, C. H., Zakaria, S., & Akil, H. M. (2016). Characterization of nanocellulose recovery from *Elaeis guineensis* frond for sustainable development. *Clean Technologies and Environmental Policy*, 18(8), 2503–2512.
- Mohamad, N., Buang, N. A., Mahat, N. A., Jamalis, J., Huyop, F., Aboul-Enein, H. Y., & Wahab, R. A. (2015a). Simple adsorption of *Candida rugosa* lipase onto multi-walled carbon nanotubes for sustainable production of the flavor ester geranyl propionate. *Journal of Industrial and Engineering Chemistry*, 32, 99–108.
- Mohamad, N. R., Marzuki, N. H. C., Buang, N. A., Huyop, F., & Wahab, R. A. (2015b). An overview of technologies for immobilization of enzymes and surface analysis techniques for immobilized enzymes. *Biotechnology, Biotechnological*

- Equipment*, 29(2), 205–220.
- Mondal, K., & Sharma, A. (2016). Recent advances in electrospun metal-oxide nanofiber based interfaces for electrochemical biosensing. *RSC Advances*, 6(97), 94595–94616.
- Mood, S. H., Golfeshan, A. H., Tabatabaei, M., Jouzani, G. S., Najafi, G. H., Gholami, M., & Ardjmand, M. (2013). Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. *Renewable and Sustainable Energy Reviews*, 27, 77–93.
- Moriana, R., Vilaplana, F., & Ek, M. (2016). Cellulose nanocrystals from forest residues as reinforcing agents for composites: a study from macro-to nano-dimensions. *Carbohydrate Polymers*, 139, 139–149.
- Mubarak, N. M., Wong, J. R., Tan, K. W., Sahu, J. N., Abdullah, E. C., Jayakumar, N. S., & Ganesan, P. (2014). Immobilization of cellulase enzyme on functionalized multiwall carbon nanotubes. *Journal of Molecular Catalysis B: Enzymatic*, 107, 124–131.
- Nagalakshmaiah, M. Melt processing of cellulose nanocrystals: thermal, mechanical and rheological properties of polymer nanocomposites. Master Thesis. Université Grenoble Alpes; 2016.
- Nardini, M., & Dijkstra, B. W. (1999). α/β hydrolase fold enzymes: the family keeps growing. *Current Opinion in Structural Biology*, 9(6), 732–737.
- Newman, R. H., & Hemmingson, J. A. (1995). Carbon-13 NMR distinction between categories of molecular order and disorder in cellulose. *Cellulose*, 2(2), 95–110.
- Ng, C. H., & Yang, K. L. (2016). Lipase in biphasic alginate beads as a biocatalyst for esterification of butyric acid and butanol in aqueous media. *Enzyme and Microbial Technology*, 82, 173–179.
- Noor, M. M. (2003). Zero burning techniques in oil palm cultivation: an economic perspective. *Oil Palm Industry Economic Journal*, 3, 16–24.
- Norajit, K., Kim, K. M., & Ryu, G. H. (2010). Comparative studies on the characterization and antioxidant properties of biodegradable alginate films containing ginseng extract. *Journal of Food Engineering*, 98(3), 377–384.
- Nordin, N. A., Sulaiman, O., Hashim, R., & Kassim, M. H. M. (2017). Oil palm frond waste for the production of cellulose nanocrystals. *Journal of Physical Science*, 28(2), 115–126.
- Oladoja, N. A., Unuabonah, E. I., Amuda, O. S., & Kolawole, O. M. *Polysaccharides*

- as a green and sustainable resources for water and wastewater treatment.* Switzerland: Springer Nature. 2017.
- Onoja, E., Chandren, S., Razak, F. I. A., & Wahab, R. A. (2018). Enzymatic synthesis of butyl butyrate by *Candida rugosa* lipase supported on magnetized-nanosilica from oil palm leaves: process optimization, kinetic and thermodynamic study. *Journal of the Taiwan Institute of Chemical Engineers*, *91*, 105–118.
- Orive, G., Ponce, S., Hernández, R. M., Gascón, A. R., Igartua, M., & Pedraz, J. L. (2002). Biocompatibility of microcapsules for cell immobilization elaborated with different type of alginates. *Biomaterials*, *23*(18), 3825–3831.
- Osuna, Y., Sandoval, J., Saade, H., López, R. G., Martínez, J. L., Colunga, E. M., & Zon, M. A. (2015). Immobilization of *Aspergillus niger* lipase on chitosan-coated magnetic nanoparticles using two covalent-binding methods. *Bioprocess and Biosystems Engineering*, *38*(8), 1437–1445.
- Öztürk, H., Pollet, E., Phalip, V., Güvenilir, Y., & Avérous, L. (2016). Nanoclays for lipase immobilization: biocatalyst characterization and activity in polyester synthesis. *Polymers*, *8*(12), 1–17.
- Palomo, J. M., Ortiz, C., Fuentes, M., Fernandez-Lorente, G., Guisan, J. M., & Fernandez-Lafuente, R. (2004). Use of immobilized lipases for lipase purification via specific lipase–lipase interactions. *Journal of Chromatography A*, *1038*(1), 267–273.
- Paroul, N., Grzegozeski, L. P., Chiaradia, V., Treichel, H., Cansian, R. L., Oliveira, J. V., & de Oliveira, D. (2010). Production of geranyl propionate by enzymatic esterification of geraniol and propionic acid in solvent-free system. *Journal of Chemical Technology and Biotechnology*, *85*(12), 1636–1641.
- Patil, K., Chopda, M., & Mahajan, R. (2011). Lipase biodiversity. *Indian Journal of Science and Technology*, *4*(8), 971–982.
- Pedersen, M., & Meyer, A. S. (2010). Lignocellulose pretreatment severity – relating pH to biomatrix opening. *New Biotechnology*, *27*(6), 739–750.
- Peng, L., Lin, L., Zhang, J., Shi, J., & Liu, S. (2011). Solid acid catalyzed glucose conversion to ethyl levulinate. *Applied Catalysis A: General*, *397*(1–2), 259–265.
- Petersson, A. E. V, Gustafsson, L. M., Nordblad, M., Börjesson, P., Mattiasson, B., & Adlercreutz, P. (2005). Wax esters produced by solvent-free energy-efficient enzymatic synthesis and their applicability as wood coatings. *Green Chemistry*, *7*(12), 837–843.

- Phanthong, P., Ma, Y., Guan, G., & Abudula, A. (2015). Extraction of nanocellulose from raw apple stem. *Journal of the Japan Institute of Energy*, 94(8), 787–793.
- Phanthong, P., Reubroycharoen, P., Hao, X., Xu, G., Abudula, A., & Guan, G. (2018). Nanocellulose: extraction and application. *Carbon Resources Conversion*, 1(1), 32–43.
- Poddar, P. K., Gupta, A., Jamari, S. S., Kim, N. S., Khan, T. A., Sharma, S., & Aziz, M. A. A. (2015). Synthesis of nanocellulose from rubberwood fibers via ultrasonication combined with enzymatic and chemical pretreatments. *Asian Journal of Applied Sciences*, 3(5), 520–527.
- Popova, M., Szegedi, Á., Lazarova, H., Ristić, A., Kalvachev, Y., Atanasova, G., & Gläser, R. (2016). Synthesis of biomass derived levulinate esters on novel sulfated Zr/KIL-2 composite catalysts. *Microporous and Mesoporous Materials*, 235, 50–58.
- Raghavendra, T., Basak, A., Manocha, L. M., Shah, A. R., & Madamwar, D. (2013). Robust nanobioconjugates of *Candida antarctica* lipase B–multiwalled carbon nanotubes: characterization and application for multiple usages in non-aqueous biocatalysis. *Bioresource Technology*, 140, 103–110.
- Rahman, I. A., Memon, A. H., & Karim, A. T. A. (2013). Significant factors causing cost overruns in large construction projects in Malaysia. *Journal of Applied Sciences*, 13(2), 286–293.
- Rahman, I. N. A., Attan, N., Mahat, N. A., Jamalis, J., Abdul Keyon, A. S., Kurniawan, C., & Wahab, R. A. (2018). Statistical optimization and operational stability of *Rhizomucor miehei* lipase supported on magnetic chitosan/chitin nanoparticles for synthesis of pentyl valerate. *International Journal of Biological Macromolecules*, 115, 680–695.
- Rahman, I. N. A., Wahab, R. A., Mahat, N. A., Jamalis, J., Huri, M. A. M., & Kurniawan, C. (2019). Ternary blended chitosan/chitin/Fe₃O₄ Nanosupport for lipase activation and stabilization. *Arabian Journal for Science and Engineering*, 44(7), 6327–6337.
- Rambabu, N., PAnthapulakkal, S., Sain, M., & Dalai, A. K. (2016). Production of nanocellulose fibers from pinecone biomass: evaluation and optimization of chemical and mechanical treatment conditions on mechanical properties of nanocellulose films. *Industrial Crops and Products*, 83, 746–754.
- Rao, K. M., Rao, K. K., Sudhakar, P., Rao, K. C., & Subha, M. C. S. (2013). Synthesis

- and characterization of biodegradable poly (vinyl caprolactam) grafted on to sodium alginate and its microgels for controlled release studies of an anticancer drug. *Journal of Applied Pharmaceutical Science*, 3(6), 61–69.
- Rhim, J. W. (2004). Physical and mechanical properties of water resistant sodium alginate films. *LWT-Food Science and Technology*, 37(3), 323–330.
- Rodrigues, R. C., & Fernandez-Lafuente, R. (2010). Lipase from *Rhizomucor miehei* as an industrial biocatalyst in chemical process. *Journal of Molecular Catalysis B: Enzymatic*, 64(1), 1–22.
- Rovera, C., Ghaani, M., Santo, N., Trabattoni, S., Olsson, R. T., Romano, D., & Farris, S. (2018). Enzymatic hydrolysis in the green production of bacterial cellulose nanocrystals. *ACS Sustainable Chemistry and Engineering*, 6(6), 7725–7734.
- Ruan, C. Q., Gustafsson, S., Strømme, M., Mihranyan, A., & Lindh, J. (2017). Cellulose nanofibers prepared via pretreatment based on Oxone[®] oxidation. *Molecules*, 22(12), 2177.
- Saba, N., Tahir, P., & Jawaid, M. (2014). A review on potentiality of nano filler/natural fiber filled polymer hybrid composites. *Polymers*, 6(8), 2247–2273.
- Sabbagh, F., Mahmoudi Khatir, N., Khodaeyan Karim, A., Omidvar, A., Nazari, Z., & Jaber, R. (2019). Mechanical properties and swelling behavior of acrylamide hydrogels using montmorillonite and kaolinite as clays. *Journal of Environmental Treatment Techniques*, 7(2), 211–219.
- Sacui, I. A., Nieuwendaal, R. C., Burnett, D. J., Stranick, S. J., Jorfi, M., Weder, C., & Gilman, J. W. (2014). Comparison of the properties of cellulose nanocrystals and cellulose nanofibrils isolated from bacteria, tunicate, and wood processed using acid, enzymatic, mechanical, and oxidative methods. *ACS Applied Materials and Interfaces*, 6(9), 6127–6138.
- Sain, M., & Panthapulakkal, S. (2006). Bioprocess preparation of wheat straw fibers and their characterization. *Industrial Crops and Products*, 23(1), 1–8.
- Saito, T., & Isogai, A. (2004). TEMPO-mediated oxidation of native cellulose: the effect of oxidation conditions on chemical and crystal structures of the water-insoluble fractions. *Biomacromolecules*, 5(5), 1983–1989.
- Saliccioli, M., Stamatakis, M., Caratzoulas, S., & Vlachos, D. G. (2011). A review of multiscale modeling of metal-catalyzed reactions: mechanism development for complexity and emergent behavior. *Chemical Engineering Science*, 66(19), 4319–4355.

- Saliluddin, S. M. (2015). Trans-boundary haze: the annual exo-“dust”. *International Journal of Public Health and Clinical Sciences*, 2(5), 1–9.
- Sánchez, R., Rodríguez, A., García, J. C., Rosal, A., & Jiménez, L. (2011). Exploitation of hemicellulose, cellulose and lignin from *Hesperaloe funifera*. *Bioresource Technology*, 102(2), 1308–1315.
- Schuchardt, U., Sercheli, R., & Vargas, R. M. (1998). Transesterification of vegetable oils: a review . *Journal of the Brazilian Chemical Society*, 9, 199–210.
- Segal, L., Creely, J. J., Martin Jr, A. E., & Conrad, C. M. (1959). An empirical method for estimating the degree of crystallinity of native cellulose using the x-ray diffractometer. *Textile Research Journal*, 29(10), 786–794.
- Seth, S., Chakravorty, D., Dubey, V. K., & Patra, S. (2014). An insight into plant lipase research – challenges encountered. *Protein Expression and Purification*, 95, 13–21.
- Shafizah, S., Izwan, A. R. S., Fatirah, F., & Hasraf, M. N. N. Review on cellulose nanocrystals (CNCs) as reinforced agent on electrospun nanofibers: mechanical and thermal properties. *The International Fundamentum Sciences Symposium*. June 25–26, 2018. Terengganu, Malaysia: IOP. 2018. 1–6.
- Shak, K. P. Y., Pang, Y. L., & Mah, S. K. (2018). Nanocellulose: recent advances and its prospects in environmental remediation. *Beilstein Journal of Nanotechnology*, 9, 2479–2498.
- Shankar, S., & Rhim, J. W. (2016). Preparation of nanocellulose from micro-crystalline cellulose: The effect on the performance and properties of agar-based composite films. *Carbohydrate Polymers*, 135, 18–26.
- Sharma, R., Chisti, Y., & Banerjee, U. C. (2001). Production, purification, characterization, and applications of lipases. *Biotechnology Advances*, 19(8), 627–662.
- Sharma, S., & Kanwar, S. (2014). Organic solvent tolerant lipases and applications. *The Scientific World Journal*, 2014, 1–15.
- Shaya, M., Mat, U. W., Yussuf, A. A., & Navid, N. (2011). Preparation and thermal properties of cellulose/layered silicate montmorillonite nanocomposites prepared via ionic liquids. *Key Engineering Materials*, 471–472, 786–791.
- Shen, X. J., Huang, P. L., Chen, J. H., Wu, Y. Y., Liu, Q. Y., & Sun, R. C. (2017). Comparison of acid-hydrolyzed and TEMPO-oxidized nanocellulose for reinforcing alginate fibers. *BioResources*, 12(4), 8180–8198.

- Shi, J., Wang, X., Zhang, S., Tang, L., & Jiang, Z. (2016). Enzyme-conjugated ZIF-8 particles as efficient and stable pickering interfacial biocatalysts for biphasic biocatalysis. *Journal of Materials Chemistry B*, 4(15), 2654–2661.
- Shuit, S. H., Tan, K. T., Lee, K. T., & Kamaruddin, A. H. (2009). Oil palm biomass as a sustainable energy source: a malaysian case study. *Energy*, 34(9), 1225–1235.
- Siqueira, P., Siqueira, É., de Lima, A., Siqueira, G., Pinzon, A., Lopes, A., & Botaro, V. (2019). Three-dimensional stable alginate-nanocellulose gels for biomedical applications: towards tunable mechanical properties and cell growing. *Nanomaterials*, 9(1), 1–22.
- Skoronski, E., Padoin, N., Soares, C., & Furigo Jr, A. (2014). Stability of immobilized rhizomucor miehei lipase for the synthesis of pentyl octanoate in a continuous packed bed bioreactor. *Brazilian Journal of Chemical Engineering*, 31(3), 633–641.
- Sun, R. C., Tomkinson, J., Wang, Y. X., & Xiao, B. (2000). Physico-chemical and structural characterization of hemicelluloses from wheat straw by alkaline peroxide extraction. *Polymer*, 41(7), 2647–2656.
- Sundarrajan, P., Eswaran, P., Marimuthu, A., Subhadra, L. B., & Kannaiyan, P. (2012). One pot synthesis and characterization of alginate stabilized semiconductor nanoparticles. *Bulletin of the Korean Chemical Society*, 33(10), 3218–3224.
- Tang, X., Sun, Y., Zeng, X., Hao, W., Lin, L., & Liu, S. (2014). Novel process for the extraction of ethyl levulinate by toluene with less humins from the ethanolysis products of carbohydrates. *Energy and Fuels*, 28(7), 4251–4255.
- Thakur, S. (2012). Lipases, its sources, properties and applications: a review. *International Journal of Scientific and Engineering Research*, 3(7), 1–29.
- Theagarajan, R., Dutta, S., Moses, J. A., & Chinnaswamy, A. *Alginates for Food Packaging Applications*. United States: John Wiley & Sons Inc. 2019.
- Thomas, B., Raj, M. C., Joy, J., Moores, A., Drisko, G. L., & Sanchez, C. (2018). Nanocellulose, a versatile green platform: From biosources to materials and their applications. *Chemical Reviews*, 118(24), 11575–11625.
- Torres, J. A., Silva, M. C., Lopes, J. H., Nogueira, A. E., Nogueira, F. G. E., & Corrêa, A. D. (2018). Development of a reusable and sustainable biocatalyst by immobilization of soybean peroxidase onto magnetic adsorbent. *International Journal of Biological Macromolecules*, 114, 1279–1287.

- Tripathi, R., & Mishra, B. (2012). Development and evaluation of sodium alginate-polyacrylamide graft-co-polymer-based stomach targeted hydrogels of famotidine. *AAPS PharmSciTech*, *13*(4), 1091–1102.
- Uyama, H., Kuwabara, M., Tsujimoto, T., Nakano, M., Usuki, A., & Kobayashi, S. (2003). Green nanocomposites from renewable resources: plant oil– clay hybrid materials. *Chemistry of Materials*, *15*(13), 2492–2494.
- Vakhlu, J., & Kour, A. (2006). Yeast lipases: enzyme purification, biochemical properties and gene cloning. *Electronic Journal of Biotechnology*, *9*(1), 1–17.
- van Pouderooyen, G., Eggert, T., Jaeger, K.-E., & Dijkstra, B. W. (2001). The crystal structure of Bacillus subtilis lipase: a minimal α/β hydrolase fold enzyme. *Journal of Molecular Biology*, *309*(1), 215–226.
- Vanamudan, A., Bandwala, K., & Padmaja, P. (2014). Adsorption property of rhodamine 6G onto chitosan-g-(N-Vinyl pyrrolidone)/montmorillonite composite. *International Journal of Biological Macromolecules*, *69*, 506–513.
- Velasco-Lozano, S., López-Gallego, F., Mateos-Díaz, J., & Favela-Torres, E. (2016). Cross-linked enzyme aggregates (CLEA) in enzyme improvement – a review. *Biocatalysis*, *1*, 166–177.
- Wagner, J. R., Mount, E. M., & Giles, H. F. Design of Experiments. In: Giles, H. F., & Wagner, J. R. ed. *Plastics Design Library*. New York: Elsevier Inc. 291–308; 2014.
- Wang, J., Liu, Y., Tang, L., Ren, X., Zeng, G., & Zhu, J. Mesoporous carbon-based enzyme biocatalyst for aquatic recalcitrant pollutant treatment. In: Tang, L., Deng, Y., Wang, J., Wang, J., & Zeng, G. ed. *Micro and Nano Technologies*. China: Elsevier Inc. 103–124; 2019.
- Wang, Y., Wei, X., Li, J., Wang, Q., Wang, F., & Kong, L. (2013). Homogeneous isolation of nanocellulose from cotton cellulose by high pressure homogenization. *Journal of Materials Science and Chemical Engineering*, *1*(5), 49–52.
- Whitford, W. G., Lundgren, M., & Fairbank, A. (2018). Cell culture media in bioprocessing. In: Jagschies, G., Lindskog, E., Łacki, K., & Galliher, P. ed. *Development, Design, and Implementation of Manufacturing Processes*. United States: Elsevier Inc. 147–162; 2018.
- Woehl, M. A., Canestraro, C. D., Mikowski, A., Sierakowski, M. R., Ramos, L. P., & Wypych, F. (2010). Bionanocomposites of thermoplastic starch reinforced with

- bacterial cellulose nanofibres: effect of enzymatic treatment on mechanical properties. *Carbohydrate Polymers*, 80(3), 866–873.
- Wonnice Ma, I., Shafaamri, A., Kasi, R., Zaini, F., Balakrishnan, V., Subramaniam, T. R., & Arof, A. K. (2017). Anticorrosion properties of epoxy/nanocellulose nanocomposite coating. *Bioresources*, 12, 2912–2929.
- Wu, C., Zhou, G., Jiang, X., Ma, J., Zhang, H., & Song, H. (2012). Active biocatalysts based on *Candida rugosa* lipase immobilized in vesicular silica. *Process Biochemistry*, 47(6), 953–959.
- Wulandari, W. T., Rochliadi, A., & Arcana, I. M. Nanocellulose prepared by acid hydrolysis of isolated cellulose from sugarcane bagasse. *10th Joint Conference on Chemistry*. September 8–9, 2015. Solo, Indonesia: IOP. 2016. 1–6.
- Yang, W., Feng, Y., He, H., & Yang, Z. (2018). Environmentally-friendly extraction of cellulose nanofibers from steam-explosion pretreated sugar beet pulp. *Materials*, 11(7), 1160.
- Yasim-Anuar, T. A. T., Ariffin, H., & Hassan, M. A. Characterization of cellulose nanofiber from oil palm mesocarp fiber produced by ultrasonication. *The Wood and Biofiber International Conference (WOBIC 2017)*. November 21–23, 2017. Selangor, Malaysia: IOP. 1–11. 2018.
- Yu, H., Qin, Z., Liang, B., Liu, N., Zhou, Z., & Chen, L. (2013). Facile extraction of thermally stable cellulose nanocrystals with a high yield of 93% through hydrochloric acid hydrolysis under hydrothermal conditions. *Journal of Materials Chemistry A*, 1(12), 3938–3944.
- Yu, P., Wang, H.-Q., Bao, R.-Y., Liu, Z., Yang, W., Xie, B. H., & Yang, M. B. (2017). Self-assembled sponge-like chitosan/reduced graphene oxide/montmorillonite composite hydrogels without cross-linking of chitosan for effective Cr(VI) sorption. *ACS Sustainable Chemistry & Engineering*, 5(2), 1557–1566.
- Zaidan, U. H., Rahman, M. B. A., Othman, S. S., Basri, M., Abdulmalek, E., Rahman, R. N. Z. R. A., & Salleh, A. B. (2012). Biocatalytic production of lactose ester catalysed by mica-based immobilised lipase. *Food Chemistry*, 131(1), 199–205.
- Zhang, D. H., Peng, L. J., Wang, Y., & Li, Y. Q. (2015). Lipase immobilization on epoxy-activated poly (vinyl acetate-acrylamide) microspheres. *Colloids and Surfaces B: Biointerfaces*, 129, 206–210.
- Zhang, D., Zhang, Q., Gao, X., & Piao, G. (2013). A nanocellulose polypyrrole composite based on tunicate cellulose. *International Journal of Polymer Science*,

- 2013, 1–6.
- Zhang, W. W., Jia, J. Q., Wang, N., Hu, C. L., Yang, S. Y., & Yu, X. Q. (2015). Improved activity of lipase immobilized in microemulsion-based organogels for (R,S)-ketoprofen ester resolution: long-term stability and reusability. *Biotechnology Reports*, 7, 1–8.
- Zhang, Z., Ortiz, O., Goyal, R., & Kohn, J. (2014). Biodegradable polymers. In: Lanza, R., Langer, R., & Vacanti, J. ed. *Principles of Tissue Engineering*. United Kingdom: Elsevier Inc. 441–473. 2014.
- Zhao, G., Liu, M., Xia, X., Li, L., & Xu, B. (2019). Conversion of furfuryl alcohol into ethyl levulinate over glucose-derived carbon-based solid acid in ethanol. *Molecules*, 24(10), 1–9.
- Zhao, R., Torley, P., & Halley, P. (2008). Emerging biodegradable materials: starch- and protein-based bio-nanocomposites. *Journal of Materials Science*, 43(9), 3058–3071.
- Zhong, T., Oporto, G. S., Jaczynski, J., & Jiang, C. (2015). Nanofibrillated cellulose and copper nanoparticles embedded in polyvinyl alcohol films for antimicrobial applications. *BioMed Research International*, 2015, 1–8.
- Zhou, Y. M., Fu, S. Y., Zheng, L. M., & Zhan, H. Y. (2012). Effect of nanocellulose isolation techniques on the formation of reinforced poly (vinyl alcohol) nanocomposite films. *Express Polymer Letters*, 6(10), 794–804.
- Zucca, P., Fernandez-Lafuente, R., & Sanjust, E. (2016). Agarose and its derivatives as supports for enzyme immobilization. *Molecules*, 21(11), 1–25.

LIST OF PUBLICATIONS

Journal of Impact Factor

1. **Hussin, F. N. N. M.**, Attan, N., and Wahab, R. A. (2020). Taguchi Design-assisted immobilization of *Candida rugosa* lipase onto a ternary alginate/nanocellulose/montmorillonite composite: Physicochemical characterization, thermal stability and reusability studies. *Enzyme and Microbial Technology*, 136, 109506. (Elsevier IF 3.553, WOS/SCOPUS – Q2)
2. **Hussin, F. N. N. M.**, Attan, N., and Wahab, R. A. (2020). Extraction and characterization of nanocellulose from raw oil palm leaves (*Elaeis guineensis*). *Arabian Journal for Science and Engineering*, 45, 175-186. (Springer IF 1.518, WOS/SCOPUS – Q3)

Non-Indexed Conference Proceeding

1. **Hussin, F. N. N. M.**, Wahab, R. A., and Attan, N. (2018). Extraction and characterization of nanocellulose from oil palm biomass. International Graduate Conference on Engineering, Science and Humanities (7th IGCESH 2018), Faculty of Built Environment and Surveying, Universiti Teknologi Malaysia.