OPTIMISATION OF BIOPULPING PROCESS BY BACTERIA FROM *RHYNCHOPHORUS FERRUGINEUS* ON EMPTY FRUIT BUNCH FOR PULP INDUSTRY

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ABSTRACT

Lignin removal is an essential phase in pulping process. The conventional pulping process has many challenges such as high chemical and energy consumptions, as well as long period and sensitivity of fungal during fermentation process. A cleaner, cheaper, and more effective lignin removal method attracts the interest of industrialists. This research focuses on optimising lignin removal via biopulping process using several combinations of *R. ferrugineus's* gut microbiome such as *Klebsiella pneumoniae* (K), *Serratia marcescens* (S), *Pseudomonas citronellolis* (P) and *Enterobacter oryzae* (E). The optimum conditions of biopulping process were determined through the design of experiment (DOE). DOE involves two phases: screening the significant parameter using Plackett-Burman design (PBD) and optimising conditions for biopulping process using Box-Behnken design (BBD). The chemical properties of EFB biopulp were characterised according to the Technical Association Pulp and Paper Industry (TAPPI), Chlorite and Kursher-Hoffner methods. On the application side, the handsheets produced were assessed on its physical and mechanical properties according to TAPPI methods. The results revealed that the quadruple culture (KSPE) combination having the highest capacity to degrade lignin by 61.86% (using alkali lignin) with the production of lignin degradation enzymes at 2230.10 U/mL (LiP), 314.84 U/mL (MnP) and 973.80 U/mL (Lac). The biopulping of EFB using KSPE combination identified the optimised conditions as follows; incubation time = 48 h, temperature = 35 °C and glucose load = 5 mL per 100 mL medium with 52.70% of lignin removal. Significantly, the cellulose, hemicellulose, lignin, and extractive contents of the biopulp were recovered at 47.37%, 31.36%, 12.70% and 1.77% respectively. The impact of percentage lignin being removed was reflected on the quality of pulp produced. In this case, the brightness (32.50%), tensile index (9.65 Nm/g), burst index (0.98 $kPa.m^2/g$) and tear index (2.71 $mN.m^2/g$) of handsheet produced were acceptable for the production of printing and writing paper grades. This study had successfully demonstrated the optimised biopulping process of KSPE microbes on EFB. The

alternative approach of delignification could promote an effective and greener technology for the future of pulp and paper industries.

ABSTRAK

Penyingkiran lignin adalah fasa penting dalam proses pulpa. Proses pulpa konvensional mempunyai banyak cabaran seperti penggunaan bahan kimia dan tenaga yang tinggi, serta jangka masa panjang dan kepekaan kulat semasa proses penapaian. Kaedah penyingkiran lignin yang lebih bersih, lebih murah, dan lebih berkesan menarik minat para perindustrian. Penyelidikan ini memfokuskan pada mengoptimumkan penyingkiran lignin melalui proses bio pulpa menggunakan beberapa kombinasi mikrob usus *Rhynchophorus ferrugineus* seperti *Klebsiella pneumoniae* (K), *Serratia marcescens* (S), *Pseudomonas citronellolis* (P), and *Enterobacter oryzae* (E). Keadaan optimum proses bio pulpa ditentukan melalui reka bentuk experiment (DOE). DOE melibatkan dua fasa: menyaring parameter penting menggunakan reka bentuk Plackett-Burman (PBD) dan mengoptimumkan keadaan untuk proses bio pulpa meggunakan reka bentuk Box-Behnken (BBD). Sifat kimia bio pulpa EFB dicirikan mengikut kaedah Teknik Industri Pulpa dan Kertas (TAPPI) dan kaedah klorit dan Kursher-Hoffner. Dari sisi aplikasi, helaian tangan (kertas) yang dihasilkan dinilai berdasarkan sifat fizikal dan mekanikalnya mengikut kaedah TAPPI. Hasilnya menunjukkan bahawa kombinasi kultur empat kali ganda (KSPE) yang mempunyai kapasiti tertinggi untuk menurunkan lignin sebanyak 61.86% (menggunakan alkali lignin) dengan pengeluaran enzim degradasi lignin pada 2230.10 U/mL (LiP), 314.84 U/mL (MnP) dan 973.80 U/mL (Lac). Bio pulpa EFB menggunakan kombinasi KSPE mengenal pasti keadaan dioptimumkan seperti berikut: masa inkubasi = 48 jam, suhu = 35 °C dan beban glukosa = 5 mL setiap 100 mL medium dengan 52.70% penyingkiran lignin. Secara ketara, kandungan selulosa, hemiselulosa, lignin dan ekstraktif bio pulpa masing-masing dipulihkan pada 47.37%, 31.36%, 12.70% and 1.77%. Kesan peratusan lignin yang dikeluarkan dicerminkan pada kualiti pulpa yang dihasilkan. Dalam kes ini, kecerahan (32.50%), indeks tegangan (9.65 Nm/g), indeks pecahan (0.98 kPa.m^2/g) and indeks koyakan (2.71 mN.m^2/g) kertas yang dihasilkan dapat diterima untuk pengeluaran mencetak dan menulis gred kertas. Kajian ini telah berjaya menunjukkan proses bio pulpa

mikrob KSPE yang dioptimumkan pada EFB. Pendekatan alternatif delignifikasi dapat mempromosikan teknologi yang efektif dan lebih hijau untuk masa depan industri pulpa dan kertas.

.

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LIST OF SYMBOLS AND ABBREVIATIONS

CFU/ mL	-	Cloning-forming unit per millilitre
cm	-	Centimetre
GJ/t	-	Gigajoules per tonne
g/m^2	-	Gram per metre square
g	-	Gram
h	-	Hour
ha	-	Hectare
IU/L	-	International units per litre
in	-	Inch
kg/m	-	Kilogram per metre
kN/m	-	Kilonewton per metre
kPa	-	Kilopascal
$kPa.m^2/g$	-	Kilopascal-metre square per gram
kW	-	Kilowatt
kWh/t	-	kilowatt hour per tonne
lb/in	-	Pound per inch
M	-	Molar
MWh	-	Megawatt-hour
min	-	Minute
mm	-	Millimetre
mL	-	Millilitre
mM	-	Millimolar
mNm^2/g	-	Millinewton metre square per gram
N/m	-	Newton per metre
Nm/g	-	Newton metre per gram
nm	-	Nanometre

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APPENDIX C

PUBLICATIONS, PROCEEDINGS AND AWARDS

C1 List of publications

1. Syarifah, S. M., Kassim, A. S. M., **Aripin, A. M.**, Chan, C. M., Zainulabidin, M. H., Ishak, N. & Siswanto, W. A. (2021). Brief dataset on produced handsheet from oil pam residue lignocellulose treated with *Bacillus cereus* on mechanical and physical characterization. *Data in Brief*, 36, 107030. (**Scopus-indexed, Q2**).
2. Syarifah, S. M., **Aripin, A. M**., Fadilat, A. and Kassim, A. S. M. (2020). Lignocellulose chemical composition and handsheet surface morphology analysis on oil palm residue biodelignification using *Bacillus cereus* from *Coptotermus curvignathus*. (2020). *SSRG International Journal of Enginnering Trends and Technology*, 68(12), 99 – 107. **ISSN**: 2231 – 5381. (**Scopus-indexed**).
3. **Aripin, A. M**., Abd Rashid, A. A., Syarifah, S. M., Ming, C. C., Zainulabidin, M. H., Ishak, N., Adnan, S., Chye, D. L. S. and Kassim, A. S. M. (2020). Oil palm empty fruit bunch (OPEFB) handsheet production from optimized biodelignification of *Rhyncophorus ferrugineus* microbiome's enzymes. *International Journal of Emerging Trends in Engineering Research*, 8(1.2), 253 – 263. **ISSN:** 2347 – 3983. (**Scopus-indexed**).
4. Ayeronfe, F., Kassim, A., Hung, P., Ishak, N., Syarifah, S. and **Aripin, A**. (2019). Evaluation of *Lysinibacillus* SP, isolated from *Coptotermers curvignathus* gut, for the delignification of oil palm residues. *Bioscience Journal*, 35(3), 892 – 902. **ISSN**: 19813163, 15163725. (**Scopus-indexed**).
5. Ayeronfe, F., Kassim, A., Hung, P., Ishak, N., Syarifah, S. and **Aripin, A**. (2019). Production of ligninolytic enzymes by *Coptotermes curvignathus* gut bacteria. *Environmental and Climate Technologies*, 23(1), 111 – 121. **ISSN**: 2255-8837. (**Scopus-indexed**).

6. Ishak. N., Yusop, N. M., Kassim, A. S. M., **Aripin, A. M**., Sharifah, S. M. and Oluwatosin, A. F. (2019). Insect enzymes for Biotechnology Industry: From nature to industrial application. *Journal of Engineering and Health Sciences*, 3 (1), 25 – 40. **ISSN**: 2600-8378.

7. Ishak. N., Kassim, A. S. M., **Aripin, A. M**., Sharifah, S. M. and Oluwatosin, A. F. (2019). A review of lignin and biodelignification. *Journal of Engineering and Health Sciences*, 3 (1), 41 – 72. **ISSN**: 2600-8378.

8. Syarifah, S. M., Kassim, A. S. M., **Aripin, A. M**., Ishak, N., Fadilat, A. and Adnan, S. (2019). Bio-mechanical pulping of bacteria pre-treatment on oil palm biomass for handsheet production. *International Journal of Engineering & Technology*, 8(1.1), 177 – 183. **ISSN:**2227-524X.

9. Ishak. N., Kassim, A. S. M., **Aripin, A. M**., Sharifah, S. M. and Oluwatosin, A. F. (2018). Differential activity of peroxidases and laccase of gut isolated strains in the changing of temperature and pH. *Journal of Engineering and Health Sciences*, 2(1), 51 – 66. **ISSN**: 2600-8378.

10. Ayeronfe, F., Kassim, A., Ishak, N., **Aripin, A**., Hung, P. and Abdulkareem, M. (2018). A review on microbial degradation of lignin. *Advanced Science Letters*, 24 (6), Number 6, 4407 – 4413. **ISSN**: 1936-6612.

11. Nordin, M. N. A. A., Wan, L. M., Zainulabidin, M. H., Kassim, A. S. M. and **Aripin, A. M.** (2016). Research finding in natural fibers sound absorbing material. *ARPN Journal of Engineering and Applied Sciences*, 14, 8579 - 8584. **ISSN**: 1819-6608. (**Scopus-indexed**).

12. Wan, L. M., Azmi, M. N., Zainulabidin, M. H., Kassim, A. S. M. and **Aripin, A. M.** (2016). Framework study of acoustical characteristics of reinforced natural fibers. *ARPN Journal of Engineering and Sciences*, 14, 8573 – 8578. **ISSN**: 1819-6608. (**Scopus-indexed**).

13. Kassim, A. S. M., **Aripin, A**. **M**., Ishak, N., Zainulabidin, M. H. and Abang Zaidel, D. N. F. (2016). Oil palm leaf fibre and its suitability for paper-based products. *ARPN Journal of Engineering and Applied Science*, 11, 7364 – 7369. **ISSN**: 1819-6608. (**Scopus-indexed**).

14. Kassim, A. S. M, **Aripin, A**. **M**, Ishak, N., Hairon, N. H., Fauzi, N. A., Razali, N. F. and Zainulabidin, M. H. (2016). Potential of cogon grass (*Imperata cylindrical*) as an alternative fibre in paper-based industry. *ARPN*

Journal of Engineering and Applied Science, 4, 2681 – 2686. **ISSN**: 1819-6608. (**Scopus-indexed**).

15. Ishak, N., Kassim, A. S. M., **Aripin, A**. **M**., Abang Zaidel, D. N. F. and Zainulabidin, M. H. (2015). Identification and expression of ligninase enzyme from tropical Asia wood insect for agro-pulp biodelignification: A theoretical framework. *Applied Mechanics and Materials*, 773-774, 1380-1383. **ISBN**: 1662-7482.
16. Kassim, A. S. M., **Aripin, A**. **M.**, Ishak, N. and Zainulabidin, M. H. (2015). Cogon grass as an alternative fibre for pulp and paper–based industry: On chemical and surface morphological properties. *Applied Mechanic and Materials*, 773-774, 1242 – 1245. **ISBN**: 1662-7482.

C2 List of proceedings:

1. **Aripin. A. M**., Kassim, A. S. M., Ishak, N., Sharifah, S. M., Ayeronfe, F. and Nayan, N. H. M. (2019). Optimisation of lignin degradation using co-culture bacterial gut species. 5th International Conference on the Application of Science and Mathematics 2019 (SCIEMATHIC 2019), Malacca, Malaysia, 14th – 15th August 2019. **Paper in progress for publication.**
2. Kassim, A. S. M., Ishak, N., **Aripin, A. M.** and Abang Zaidel, D. N. F. (2016). Potential lignin degraders isolated from the gut of *Rhynchophorous Ferrugineus*. Advance of Engineering Research, International Conference on Mechanics, Materials and Structural Engineering (ICMMSE), Atlantis press, 66 – 72. ISSN: 2352-5401.

C3 List of awards

1. **Silver Medal** of Application of bacteria in pulp production on oil palm biomass residue in the **FTK Research & Innovative Technology Competition 2018** (RITEC 2018) Universiti Tun Hussein Onn Malaysia, Pagoh, Muar, Johor, Malaysia.

2. **Silver Medal** of Insect's enzyme for biotechnology industry in the **Research and Innovation Festival 2016** on 14th – 15th November 2016 at Universiti Tun Hussein Onn Malaysia, Johor, Malaysia.
3. **First Prize Winner for Best Paper Award**, Chemical Engineering and Natural Resources (CHEM) on *Potential of cogon grass (Imperata cylindrical) as an alternative fiber in paper-based industry* in the **Malaysian Technical Universities Conference on Engineering and Technology** (MUCET) on 11th - 13th October 2015 at Johor Bahru, Malaysia.
4. **Best of the Best Paper Award** for paper entitled "*Potential of cogon grass (Imperata cylindrical) as an alternative fiber in paper-based industry*" in **the Malaysian Technical Universities Conference on Engineering and Technology** (MUCET) on 11th - 13th October 2015 at Johor Bahru, Malaysia.