

DISERTASI

**POTENSI ENZIM SELULASE HASIL ISOLASI DARI
BAKTERI SELULOLITIK DI DALAM SALURAN
PENCERNAAN RAYAP LAHAN GAMBUT**

**Diajukan untuk memenuhi salah satu syarat memperoleh gelar
Doktor Ilmu MIPA pada Universitas Sriwijaya**



DWITA OKTIARNI

08013681924004

**PROGRAM STUDI DOKTOR ILMU MIPA
FAKULTAS MATEMATIKA DAN ILMU PENGETAHUAN ALAM
UNIVERSITAS SRIWIJAYA**

2022

HALAMAN PENGESAHAN

**POTENSI ENZIM SELULASE HASIL ISOLASI DARI BAKTERI
SELULOLITIK DI DALAM SALURAN PENCERNAAN
RAYAP LAHAN GAMBUT**

DISERTASI

Diajukan untuk memenuhi salah satu syarat memperoleh gelar
Doktor Ilmu MIPA pada Universitas Sriwijaya

Oleh
DWITA OKTIARNI
NIM 08013681924004

Telah disetujui,

Pada tanggal.....

**Pembimbing
Promotor**



Prof. Hermansyah, S.Si., M.Si., Ph. D
NIP 197111191997021001

Co. Promotor I



Dr. Hasanudin, S.Si., M. Si
NIP 197205151997021003

Co. Promotor II



Dr. Miksusanti, M. Si
NIP 196807231993032003

RINGKASAN

POTENSI ENZIM SELULASE HASIL ISOLASI DARI BAKTERI SELULOLITIK DI DALAM SALURAN PENCERNAAN RAYAP LAHAN GAMBUT

Dwita Oktiarni, dibimbing oleh Prof. Hermansyah, S. Si., M. Si., Ph. D.,
Dr. I Hasanudin, S. Si., M. Si., dan Dr. Miksusanti, M. Si.

Program Studi Ilmu MIPA Program Doktor
Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Sriwijaya

Bioetanol merupakan sumber energi yang mempunyai potensi untuk dikembangkan sebagai energi terbarukan dan ramah lingkungan. Beberapa negara telah mengembangkan teknologi dalam menghidrolisis biomassa selulosa untuk menghasilkan bioetanol. Produksi bioetanol dengan perlakuan pendahuluan seperti fisiko-kimia, dengan cara *steam explosion* dan *ammonia fiber explosion* cenderung menghabiskan biaya yang tinggi. Perlakuan pendahuluan dengan cara kimia, seperti hidrolisis asam dan hidrolisis alkali menghasilkan senyawa kimia yang berbahaya bagi lingkungan. Salah satu teknologi yang digunakan untuk menghasilkan bioetanol dari biomassa selulosa yaitu secara enzimatik. Hidrolisis selulosa menggunakan enzim merupakan langkah yang efektif, ramah lingkungan dan tidak menghasilkan senyawa kimia yang berbahaya bagi lingkungan. Semua industri menerapkan beberapa parameter yang harus diperhatikan, seperti menggunakan katalis enzim, ramah lingkungan dan bahan baku yang rendah, dan tidak terlalu berdampak buruk terhadap lingkungan. Biomassa lignoselulosa dari pertanian dan industri kayu dapat dimanfaatkan sebagai bahan baku yang ketersediaannya melimpah di alam. Beberapa organisme mengkonsumsi selulosa sebagai sumber makanannya, salah satunya rayap. Rayap yang hidup berkoloni, merupakan hama bagi petani sawit maupun karet, dikarenakan dapat merusak tanaman tersebut dengan bersarang di dalam pohon atau pun membuat sarang di dalam tanah dan gundukan tanah. Namun, rayap merupakan faktor utama dari sirkulasi jaringan makanan pada lahan gambut tropis dan berperan dalam menjaga stabilitas ekosistem. Rayap merupakan serangga yang terdiri dari *lower termite* dan *higher termite* yang dapat mendegradasi selulosa menjadi monosakarida, dengan bantuan enzim selulolitik yang ada dalam saluran pencernaannya. Sampel rayap pada penelitian ini diambil pada lahan tidur di Tanjung Senai, Indralaya, Ogan Ilir. Penelitian dilanjutkan dengan isolasi dan identifikasi spesies bakteri selulolitik yang ada pada saluran pencernaan rayap. Tahapan selanjutnya dilakukan isolasi, pemurnian, dan karakterisasi terhadap enzim selulase dari bakteri pada saluran pencernaan rayap tersebut. Teknik pengambilan sampel dilakukan secara langsung, dengan cara membongkar sarang rayap yang berupa gundukan tanah dan mengambil sampel rayap prajurit dan sampel rayap pekerja beserta sarangnya. Sampel rayap yang diambil sekitar 30 rayap prajurit dan 30 rayap pekerja, beserta sarangnya, sampel disimpan di dalam wadah kayu, dan langsung dibawa ke laboratorium. Sebanyak 10

sampel rayap pekerja diisolasi untuk memperoleh bakteri yang ada di dalam saluran pencernaannya. Terdapat 64 koloni bakteri setelah diisolasi pada media agar CMC, dengan 24 koloni bakteri memperlihatkan aktivitas selulolitik yang tinggi dibandingkan dengan isolat yang lainnya. Koloni bakteri yang diperoleh kemudian diidentifikasi dengan pendekatan biomolekular dan sebanyak 6 spesies bakteri berhasil diperoleh. Bakteri selulolitik tersebut diidentifikasi dari spesies *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Klebsiella quasipneumonia*, *Klebsiella variicola*, *Enterobacter roggenskampii*, dan *Enterobacter asburiae*. Salah satu spesies bakteri dengan zona hidrolisis yang tertinggi, yaitu *Klebsiella variicola*, diisolasi untuk mendapatkan enzim selulolitik pada media CMC cair. Penelitian ini dilakukan optimasi terhadap media kultur pertumbuhan untuk produksi enzim selulase, meliputi waktu inkubasi, sumber karbon dan variasi konsentrasi sumber karbon, sumber nitrogen dan variasi konsentrasi sumber nitrogen, pH, temperatur, dan kecepatan agitasi. Hasil yang diperoleh menunjukkan bahwa media pertumbuhan optimasi yang aktivitas enzim optimum terdapat pada sumber karbon: fruktosa dengan konsentrasi 3,5%, sumber nitrogen: natrium nitrat dengan konsentrasi 2%, pH 5, temperatur 45°C, dan kecepatan agitasi 150 rpm. Produksi enzim dilakukan pada media optimasi sebanyak 1 L media cair, dan diperoleh 5 mL enzim selulase. Tahap pemurnian ekstrak kasar enzim meliputi fraksinasi dengan ammonium sulfat dan dialisis, dan kemudian dipekatkan dengan *microcentrifuge filter tube* sampai diperoleh 2 mL enzim selulase. Enzim selulase dari *Klebsiella variicola* kemudian dikarakterisasi, meliputi penentuan berat molekul dengan metode SDS-PAGE dan zimografi, serta penentuan pH dan temperatur optimum enzim selulase. Hasil penelitian diperoleh bahwa enzim memiliki berat molekul sekitar 20 kDa dengan pH optimum pada pH 6 dan temperatur optimum pada 50°C.

Kata kunci: bioetanol, biomassa, enzim selulase, rayap, selulosa.

SUMMARY

THE POTENCY OF CELLULASE ENZYME ISOLATED FROM CELLULOLYTIC BACTERIA FROM TERMITE GUT

Dwita Oktiarni, supervised by Prof. Hermansyah, S. Si., M. Si., Ph. D,
Dr. Hasanudin, S. Si., M. Si., and Dr. Miksusanti, M. Si.

Doctoral Program of Mathematics and Natural Sciences
Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya

The production of second-generation bioethanol as renewable energy has developed very rapidly and has become a promising alternative energy source. Several countries have developed technology to hydrolyze cellulosic biomass to produce bioethanol. Bioethanol is an energy source that has the potential to be developed as renewable and eco-friendly energy. Bioethanol needs higher cost and produces chemical compounds that are harmful to the environment. One of the technologies used to produce bioethanol from cellulosic biomass is by enzymatic process. Hydrolysis of cellulose using enzymes is an effective step, environmentally. Bioethanol production using biomass can be obtained alternatively from cellulose in wood, sawdust, organic waste, and agricultural waste. Cellulases are enzymes capable of hydrolyzing lignocellulose into glucose. This research used termites obtained from Indralaya peatland area as organisms that can decompose cellulose into glucose with the cellulase enzymes produced by bacteria in their gut. Termites are organisms that can inflict damage on agricultural, forest, and furniture materials. Furthermore, they can positively affect the ecosystem due to their ability to break down lignocellulose-containing materials. The termite *Macrotermes gilvus* Hagen from Tanjung Senai, Indralaya peatland region, is classified as higher levels termite. Termites like eusocial insects break down cellulosic biomass into glucose by bacteria that produce cellulolytic enzymes in their gut. The study aimed to isolate and identify of cellulolytic bacteria from termite gut obtained from Indralaya peatland area. The bacterial isolates were classified by using morphological and biochemical standard methods, and identification based on Bergey's Manual of Determinative Bacteriology. Cellulolytic bacteria of termite gut were isolated and cultured on CMC (Carboxymethyl cellulose) agar medium. The activity of cellulolytic bacterial was conducted based on halo area on a CMC agar medium. Among 64 isolates of bacteria, 24 isolates were identified as cellulolytic bacteria. In the present study, new species of cellulolytic bacteria from *Macrotermes gilvus* Hagen gut were determined by biomolecular assay. Bacterial isolates were isolated and purified by using DNA kit and the concentration of DNA bacterial isolates was obtained by using spectrophotometer nanodrop. A total of 24 bacterial isolates were amplified using PCR to determine the fragment of 16S rRNA gene, performed using BLAST-N program and compared with NCBI database. Result from the sequences of 16S rRNA gene showed that the species were identify as *Enterobacter*

cloacae, *Klebsiella pneumoniae*, *Klebsiella quasipneumoniae*, *Klebsiella varicolla*, *Enterobacter roggenkampii* and *Enterobacter asburiae*. In this study, cellulolytic *Klebsiella variicola* isolated from termite gut were optimized to increase the cellulolytic enzyme production. Several factors can affect the growth and production of cellulase enzymes. In this research, optimization of the culture medium was examined using different carbon sources, various concentration of carbon sources, diverse nitrogen sources, various concentration of nitrogen sources, pH, temperature, and agitation. The result showed that medium optimal with higher enzyme activity was obtained in the addition of a carbon source of fructose with concentration of 3.5%; nitrogen source of sodium nitrate with a concentration of 2%; pII of 5; temperature of 45°C; and agitation of 150 rpm. According to previous findings, the cellulolytic bacteria from the termite *Macrotermes gilvus* gut obtained in Indralaya Peatlands showed activity and hydrolyzed cellulose on a CMC agar medium. In this study, the cellulase enzymes of *Klebsiella variicola* isolated from *Macrotermes gilvus* gut showed higher halo area after staining with Congo red. Furthermore, the enzyme was purified and characterized using ammonium sulphate precipitation and dialysis. SDS PAGE analysis reported a molecular mass of 20 kDa, with optimal activity at pH of 6 and temperature of 50°C.

Keywords: bioethanol, biomass, cellulose, cellulase enzyme, termite.

PERNYATAAN INTEGRITAS

Yang bertanda tangan di bawah ini:

Nama : Dwita Oktiarni

NIM : 08013681924004

Judul : Potensi enzim selulase hasil isolasi dari bakteri selulolitik di dalam saluran pencernaan rayap lahan gambut.

Menyatakan bahwa semua data dan informasi yang dimuat dalam disertasi ini merupakan hasil penelitian saya sendiri didampingi Promotor dan Ko-Promotor dan bukan hasil penjiplakan/plagiat. Apabila ditemukan unsur penjiplakan/plagiasi dalam disertasi ini, maka saya bersedia menerima sanksi akademik dari Universitas Sriwijaya sesuai dengan aturan yang berlaku.

Demikian pernyataan ini saya buat dalam keadaan sadar dan tanpa ada paksaan dari siapapun.

Indralaya, Nopember 2022

Dwita Oktiarni
08013681924004

RIWAYAT HIDUP

Penulis dilahirkan di Jakarta, pada tanggal 20 Oktober 1981 yang merupakan putri kedua dari bapak Drs. H. Hasan Kodri Asir dan ibu Hj. Emmy Sutima Usia Malaya. Penulis mengenyam Pendidikan pada Taman Kanak-Kanak di TK Bhayangkari di Kota Muara Enim (1985-1987), dan melanjutkan Pendidikan Sekolah Dasar pada SDN XVIII Kota Muara Enim (1987-1993). Penulis kemudian melanjutkan sekolah pada SMPN 1 Kota Muara Enim (1993-1996) dan SMAN 2 Kota Prabumulih (1996-1999). Selanjutnya penulis meneruskan sekolah pada jenjang Sarjana pada Jurusan Kimia Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Sriwijaya (1999-2004). Penulis melanjutkan studi pada Sekolah Pascasarjana Jurusan Kimia Fakultas Matematika dan Ilmu Pengetahuan Alam Institut Teknologi Bandung (2005-2008). Kemudian penulis melanjutkan studi pada Sekolah Pascasarjana Program Studi S3-Doktor Ilmu MIPA Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Sriwijaya (2019-2022). Penulis merupakan staf pengajar pada Jurusan Kimia Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Bengkulu, sejak tahun 2009 sampai dengan sekarang.

HALAMAN PERNYATAAN PERSETUJUAN PUBLIKASI KARYA ILMIAH

Yang bertanda tangan di bawah ini:

Nama : Dwita Oktiarni
NIM : 08013681924004
Judul : Potensi enzim selulase hasil isolasi dari bakteri selulolitik di dalam saluran pencernaan rayap lahan gambut.

Memberikan izin kepada Promotor dan Universitas Sriwijaya untuk mempublikasikan hasil penelitian saya untuk kepentingan akademik apabila dalam waktu 1 (satu) tahun tidak mempublikasikan karya penelitian saya. Dalam kasus ini saya setuju untuk menempatkan Promotor sebagai penulis korespondensi (*Corresponding author*).

Demikian, pernyataan ini saya buat dalam keadaan sadar dan tanpa ada paksaan dari siapapun.

Indralaya, Nopember 2022

Dwita Oktiarni
08013681924004

DAFTAR ISI

	Halaman
HALAMAN JUDUL.....	i
HALAMAN PENGESAHAN.....	ii
RINGKASAN	iii
<i>SUMMARY</i>	v
PERNYATAAN INTEGRITAS	vii
RIWAYAT HIDUP	viii
KATA PENGANTAR DAN UCAPAN TERIMA KASIH.....	ix
HALAMAN PERNYATAAN PERSETUJUAN PUBLIKASI KARYA ILMIAH....	xi
DAFTAR ISI.....	xii
DAFTAR GAMBAR	xiv
DAFTAR TABEL	xv
DAFTAR LAMPIRAN.....	xvi
DAFTAR SINGKATAN	xvii
HALAMAN PERSEMBAHAN.....	xviii
BAB 1. PENDAHULUAN	1
1.1 Latar Belakang	1
1.2 Rumusan Masalah.....	4
1.3 Hipotesis Penelitian.....	4
1.4 Tujuan Penelitian.....	4
1.5 Kebaruan Riset	5
1.6 Manfaat Penelitian	7
1.6 Kerangka Pikir	7
BAB 2. TINJAUAN PUSTAKA	8
2.1 Lignoselulosa.....	9
2.2 Hidrolisis selulosa	10
2.3 Rayap.....	12
2.4 Bakteri pada rayap penghasil enzim selulase	13
2.5 Isolasi dan identifikasi bakteri selulolitik pada rayap	14
2.6 Enzim selulase.....	17
2.7 Isolasi, pemurnian, dan identifikasi enzim selulase	19
2.8 Karakterisasi enzim selulase.....	22
2.9 Uji aktivitas enzim.....	23
BAB 3. METODE PENELITIAN.....	24
3.1 Waktu dan Tempat	24
3.1.1 Lokasi dan teknik pengambilan sampel rayap.....	24
3.2 Alat dan Bahan Penelitian	25
3.2.1 Alat	25
3.2.2 Bahan.....	26
3.3 Pemilihan dan identifikasi rayap	27
3.4 Isolasi, pemurnian, skrining, dan karakterisasi bakteri yang ada pada saluran pencernaan rayap.....	27
3.4.1 Isolasi bakteri.....	27

3.4.2 Pemurnian dan skrining isolat bakteri selulolitik	28
3.4.3 Karakterisasi isolat bakteri: Uji Biokimia	28
3.5 Identifikasi bakteri.....	29
3.5.1 Isolasi DNA.....	29
3.5.2 Amplifikasi gen 16S rRNA (Boontanom and.....	30
3.5.3 Analisis Bioinformatika dan Pohon Filogenetik	30
3.6 Isolasi, pemurnian dan karakterisasi enzim selulolitik yang diperoleh dari isolat bakteri pada saluran pencernaan rayap.....	31
3.6.1 Optimasi media kultur	31
3.6.2 Isolasi enzim.....	32
3.6.2 Elektroforesis Gel.....	33
3.6.2.1 <i>Sodium Dodecylsulfate Polyacrilamide Gel Electrophoresis</i> (SDS PAGE) ...	33
3.6.2.2 Analisis Zimografi.....	34
3.6.3 Uji aktivitas enzim selulase.....	34
3.6.5 Karakterisasi enzim	35
3.6.5.1 Penentuan kadar protein	35
3.6.5.2 Penentuan pH optimum enzim	35
3.6.5.3 Penentuan temperatur optimum enzim	36
BAB 4. HASIL DAN PEMBAHASAN.....	37
4.1 Pemilihan dan identifikasi rayap lahan gambut.....	37
4.2 Isolasi, pemurnian dan karakterisasi bakteri yang ada pada saluran pencernaan rayap	38
4.2.1 Isolasi bakteri.....	38
4.2.2 Skrining isolat bakteri selulolitik.....	39
4.2.3 Isolasi DNA isolat bakteri, dan Amplifikasi gen 16S rRNA.....	43
4.2.3.1 Isolasi DNA isolat bakteri	43
4.2.4.3 Amplifikasi gen 16S rRNA dengan PCR (<i>Polymerase Chain Reaction</i>).....	44
4.3 Isolasi, pemurnian, dan karakterisasi enzim selulolitik yang diperoleh dari isolat bakteri pada saluran pencernaan rayap.....	58
4.3.1 Optimasi media kultur	59
4.3.2 Fraksinasi enzim dengan ammonium sulfat	64
4.3.3 SDS PAGE	66
4.3.4 Penentuan pH optimum enzim	67
4.3.5 Penentuan temperatur optimum enzim	68
BAB 5. SIMPULAN DAN SARAN	70
5.1 Simpulan.....	70
5.2 Saran.....	72
DAFTAR PUSTAKA	73
DAFTAR LAMPIRAN.....	82

DAFTAR GAMBAR

	Halaman
1.1 Diagram kerangka pikir penelitian	7
2.1 Struktur molekul selulosa	9
2.2 Proses hidrolisis selulosa menjadi glukosa oleh enzim selulase	10
2.3 Rayap <i>Macrotermes gilvus</i> Hagen kasta prajurit dan pekerja	12
2.4 Mekanisme hidrolisis selulosa menjadi glukosa oleh enzim selulase ...	17
3.1 Peta pengambilan sampel rayap	24
4.1 Rayap <i>Macrotermes gilvus</i> Hagen kasta pekerja (A) dan prajurit (B)....	37
4.2 Koloni bakteri yang terdapat dalam saluran pencernaan rayap (seri pengenceran 10 ⁻³)	38
4.3 Subkultur koloni bakteri pada media agar CMC	39
4.4 Skrining isolat bakteri selulolitik dengan larutan 1% <i>Congo red</i>	40
4.5 Subkultur isolat tunggal bakteri pada media agar CMC	41
4.6 Aktivitas selulolitik isolat bakteri pada media agar CMC dengan menggunakan larutan iodin	42
4.7 Profile produk PCR fragmen gen 16S rDNA pada gel elektroforesis ...	45
4.8 Dendogram dari isolat bakteri 1, 3, 6, 7, 10, dan 14	47
4.9 Dendogram dari isolat bakteri 19, 20, 24, 25, 28, dan 29	49
4.10 Dendogram dari isolat bakteri 36, 37, 39, 40, 43, dan 44	50
4.11 Dendogram dari isolat bakteri 52, 56, 59, 60, 62, dan 64	52
4.12 Sifat Gram 24 isolat bakteri (perbesaran 1000 X)	54
4.13 Isolat bakteri <i>K. variicola</i> (a), dan zona bening hasil hidrolisis isolat bakteri dengan pewarnaan <i>Congo red</i> (b)	58
4.14 Pengaruh waktu inkubasi terhadap aktivitas enzim	59
4.15 Pengaruh penambahan sumber karbon (a) dan variasi konsentrasi fruktosa (b) terhadap aktivitas enzim selulase	60
4.16 Pengaruh penambahan sumber nitrogen (a) dan variasi konsentrasi natrium nitrat (b) terhadap aktivitas enzim selulase	61
4.17 Pengaruh perubahan pH (a) dan temperatur (b) terhadap aktivitas enzim selulase	62
4.18 18 Pengaruh variasi kecepatan agitasi terhadap aktivitas enzim selulase	64
4.19 Pengaruh fraksinasi dengan amonium sulfat terhadap aktivitas enzim selulase	64
4.20 Gel SDS PAGE (a.1) marker protein, (a.2) ekstrak kasar enzim pada fraksi ammonium sulfat, (a.3) ekstrak kasar enzim setelah didialisis, dan (b) zona bening hasil hidrolisis enzim dengan pewarnaan <i>Congo red</i>	66
4.21 Pengaruh berbagai pH terhadap aktivitas enzim selulase	67
4.22 Pengaruh perubahan temperatur terhadap aktivitas enzim selulase	69

DAFTAR TABEL

	Halaman
1.1 Perkembangan penelitian yang telah dilakukan terhadap enzim selulolitik yang ada pada bakteri di dalam saluran pencernaan rayap	5
2.1 Produksi enzim selulase oleh strain mikroba dengan sumber karbon dari limbah tanaman	18
3.1 Matriks langkah kerja terhadap keberhasilan penelitian yang dilakukan	27
4.1 Konsentrasi DNA isolat bakteri menggunakan spektrofotometer nanodrop	44
4.2 Data BLAST-N gen 16S rRNA isolat 1, 3, 6, 7, 10, dan 14	46
4.3 Data BLAST-N gen 16S rRNA isolat 19, 20, 24, 25, 28, dan 29	48
4.4 Data BLAST-N gen 16S rRNA isolat 36, 37, 39, 40, 43, dan 44	50
4.5 Data BLAST-N gen 16S rRNA isolat 52, 56, 59, 60, 62, dan 64	51
4.6 Ringkasan data BLAST-N gen 16S rRNA isolat bakteri	53
4.7 Pengamatan makroskopis dan mikroskopis isolat bakteri	55
4.8 Uji biokimia isolat bakteri	56
4.9 Tahapan pemurnian enzim dari <i>Klebsiella variicola</i>	49

DAFTAR LAMPIRAN

	Halaman
1. Skema penelitian	82
2. Tabel penelitian	83
3. Hasil determinasi sampel rayap.....	87
4. Dokumentasi penelitian.....	89
5. Hasil sekuensing isolat bakteri	91
6. Hasil BLAST isolat bakteri	101
7. Data Perhitungan Aktivitas Ekstrak Kasar Enzim	107
8. Artikel yang telah dipublikasikan	114

BAB I

PENDAHULUAN

1.1 Latar Belakang

Indonesia merupakan negara yang kaya akan biodiversitas, baik kekayaan flora maupun fauna. Letak negara Indonesia di daerah khatulistiwa menjadikannya memiliki hutan hujan tropis yang tersebar di berbagai propinsi di wilayah Indonesia. Indonesia juga memiliki lahan gambut yang luas, yang berada di pulau Sumatera dan sebagian di pulau Kalimantan. Lahan gambut di kedua pulau ini merupakan daerah perkebunan yang didominasi oleh perkebunan sawit dan perkebunan karet, namun beberapa diantaranya hanya merupakan lahan yang tidak ditanami oleh apapun (lahan tidur) (Arif *et al.*, 2019; Brune, 2014). Berbagai organisme yang hidup di lahan gambut banyak ditemukan, diantaranya rayap. Rayap yang hidup berkoloni, merupakan hama bagi petani sawit maupun karet, dikarenakan dapat merusak tanaman tersebut dengan bersarang di dalam pohon atau pun membuat sarang di dalam tanah dan gundukan tanah. Sarang rayap biasanya berada di sekitar pohon sawit/karet dan di daerah lahan tidur berupa gundukan tanah. Rayap merupakan fauna yang sumber utama makanannya diperoleh dari selulosa, yang berasal dari pohon, meliputi kayu, batang, akar, dan daun (Brune, 2014; Hongoh, 2011).

Selulosa merupakan komponen utama dari lignoselulosa. Lignoselulosa terdiri dari polimer selulosa (40-50%), hemiselulosa (25-35%), lignin (15-20%) (Iqbal *et al.*, 2013). Selulosa memiliki ikatan β -1,4 glikosidik, yang ikatannya sulit untuk dihidrolisis menjadi monomer/monosakarida yang sederhana. Hemiselulosa, seperti xilan memiliki ikatan β -1,4 glikosidik yang terikat pada manosa, galaktosa, arabinosa dan asam glukoronat. Hemiselulosa cenderung lebih mudah untuk dihidrolisis menjadi monosakarida (Li *et al.*, 2014). Lignin merupakan polimer heterogen yang ditemukan pada lignoselulosa, biasanya memiliki tiga alkohol aromatik dalam bentuk *coniferyl alcohol*, *sinupyl*, dan *p-coumaryl* (Feofilova & Mysyakina, 2016; D. Sharma *et al.*, 2015).

DAFTAR PUSTAKA

- Abou-taleb, K., & Nasr, S. (2009). Nutritional and Environmental Factors Affecting Cellulase Production by Two Strains of Cellulolytic Bacilli Nutritional and Environmental Factors Affecting Cellulase Production by Two Strains of Cellulolytic *Bacilli*. *Australian Journal of Basic and Applied Sciences*, 3(3), 2429–2346.
- Adebola, A. B., Sarafadeen, K. O., & Idowu, A. A. (2019). Optimization of cellulase enzyme from sorghum straw by yeasts isolated from plant feeding – termite *Zonocerus variegatus*. *Food and Applied Bioscience Journal*, 7(3), 81–99.
- Afzal, M., Qureshi, M. Z., Khan, S., Khan, M. I., Ikram, H., Ashraf, A., Iqbal, A., & Qureshi, N. A. (2019). Production, purification and optimization of cellulase by *Bacillus licheniformis* HI-08 isolated from the hindgut of wood-feeding termite. *International Journal of Agriculture and Biology*, 21(1), 125–134. <https://doi.org/10.17957/IJAB/15.0872>.
- Ali, H. R. K., Hemed, N. F., & Abdelaliem, Y. F. (2019). Symbiotic cellulolytic bacteria from the gut of the subterranean termite *Psammotermes hypostoma* Desneux and their role in cellulose digestion. *AMB Express*, 9(1). <https://doi.org/10.1186/s13568-019-0830-5>.
- Arif, A., Muin, M., Larekeng, S. H., & Lestari, P. I. (2019). Survey and morphological identification of termites (Insecta : Isoptera) in Teaching Forest of Hasanuddin University, Indonesia. *IOP Conf. Ser.: Earth Environ. Sci.* 270. 012001 <https://doi.org/10.1088/1755-1315/270/1/012001>.
- Arsyad, S., Wiyono, S., & Herliyana, E. N. (2018). Screening of cellulolytic bacteria originated from termite gut for decomposing. *Jurnal Silvikultur Tropika*, 09(3), 217–222.
- Ayitso, A. S., & Onyango, D. M. (2016). Isolation and Identification by Morphological and Biochemical Methods of Antibiotic Producing Microorganisms from the gut of *Macrotermes michaelseni* in Maseno, Kenya. *Journal of Applied Biology & Biotechnology*, 4(01), 027–033. <https://doi.org/10.7324/jabb.2016.40105>.
- Banaganapalli, B., Shaik, N. A., Rashidi, O. M., Jamalalail, B., Bahattab, R., Bokhari, H. A., & Alqahtani, F. (2019). Essentials of Bioinformatics (Volume I. 355–370). Springer Nature Switzerland. <https://doi.org/10.1007/978-3-030-02634-9>.
- Bano, A., Chen, X., Prasongsuk, S., Akbar, A., Lotrakul, P., Punnapayak, H., Anwar, M., Sajid, S., & Ali, I. (2019). Purification and Characterization of Cellulase from Obligate Halophilic *Aspergillus flavus* (TISTR 3637) and Its Prospects for Bioethanol Production. *Applied Biochemistry and Biotechnology*, 189(4), 1327–1337. <https://doi.org/10.1007/s12010-019-03086-y>
- Barani, K., Manipal, S., Prabu, D., Ahmed, A., Adusumilli, P., & Jeevika, C. (2014). Anti-fungal activity of *Morinda citrifolia* (noni) extracts against *Candida albicans*: An in vitro study. *Indian Journal of Dental Research*, 25(2), 188–190. <https://doi.org/10.4103/0970-9290.135918>
- Barbosa, K. L., Malta, V. R. dos S., Machado, S. S., Leal Junior, G. A., da Silva, A. P.

- V., Almeida, R. M. R. G., & da Luz, J. M. R. (2020). Bacterial cellulase from the intestinal tract of the sugarcane borer. *International Journal of Biological Macromolecules*, *161*, 441–448. <https://doi.org/10.1016/j.ijbiomac.2020.06.042>.
- Batool, I., Awais, M., & Ahmed, D. (2021). Assessment and Characterization of Xylanolytic Bacteria Isolated from the Gut of *Microtermes Obesi* for Biomass Pretreatment. 1–13.
- Batool, I., Gulfranz, M., Asad, M. J., Kabir, F., Khadam, S., & Ahmed, A. (2018). *Cellulomonas* sp. Isolated from termite gut for saccharification and fermentation of agricultural biomass. *BioResources*, *13*(1), 752–763. <https://doi.org/10.15376/biores.13.1.752-763>.
- Behera, B. C., Mishra, R. R., Singh, S. K., & Dutta, S. K. (2016). Cellulase from *Bacillus licheniformis* and *Brucella* sp. isolated from mangrove soils of Mahanadi river delta, Odisha, India. *Biocatalysis and Biotransformation*. Early online. 1–19. <https://doi.org/10.1080/10242422.2016.1212846>.
- Berkowitz, S. A. (2020). Chromatography (other than size-exclusion chromatography) and electrophoresis. In *Biophysical Characterization of Proteins in Developing Biopharmaceuticals*. Elsevier B.V. <https://doi.org/10.1016/b978-0-444-64173-1.00014-7>.
- Bhardwaj, N., Kumar, B., Agrawal, K., & Verma, P. (2021). Current perspective on production and applications of microbial cellulases : a review. *Bioresources and Bioprocessing*, *8*(95), 1–41. <https://doi.org/10.1186/s40643-021-00447-6>.
- Boontanom, P., & Chantarasiri, A. (2021). Diversity and cellulolytic activity of culturable bacteria isolated from the gut of higher termites (*Odontotermes* sp.) in eastern Thailand. *Biodiversitas*, *22*(8), 3349–3357. <https://doi.org/10.13057/bio-div/d220831>.
- Brock, T. D., & Madigan, M. T. (1988) *Biology of microorganisms*. Prentice-Hall, New Jersey.
- Brune, A. (2014). Symbiotic digestion of lignocellulose in termite guts. *Nature Reviews Microbiology*, *12*(3), 168–180. <https://doi.org/10.1038/nrmicro3182>.
- Brunelle, J. L., & Green, R. (2014). One-dimensional SDS-polyacrylamide gel electrophoresis (1D SDS-PAGE). In *Methods in Enzymology* (1st ed., Vol. 541). Elsevier Inc. <https://doi.org/10.1016/B978-0-12-420119-4.00012-4>.
- Cooney, S., O'Brien, S., Iversen, C., & Fanning, S. (2014). Bacteria: Other Pathogenic Enterobacteriaceae-*Enterobacter* and Other Genera. In *Encyclopedia of Food Safety* (Vol. 1). Elsevier Ltd. <https://doi.org/10.1016/B978-0-12-378612-8.00104-9>.
- Ding, S., Xu, Q., Crowley, M., Zeng, Y., Nimlos, M., Lamed, R., Bayer, E. A., & Himmel, M. E. (2008). A biophysical perspective on the cellulosome: new opportunities for biomass conversion. *Current Opinion in Biotechnology*, *19*, 218–227. <https://doi.org/10.1016/j.copbio.2008.04.008>.
- Duan, J., Liu, J., Ma, X., Zhang, Y., Wang, X., & Zhao, K. (2017). Isolation, identification, and expression of microbial cellulases from the gut of *Odontotermes formosanus*. *Journal of Microbiology and Biotechnology*, *27*(1), 122–129. <https://doi.org/10.4014/jmb.1604.04040>.
- Ejaz, U., Muhammad, S., Hashmi, I. A., Ali, F. I., & Sohail, M. (2020). Utilization of

- methyltrioctylammonium chloride as new ionic liquid in pretreatment of sugarcane bagasse for production of cellulase by novel thermophilic bacteria. *Journal of Biotechnology*, 317, 34–38. <https://doi.org/10.1016/j.jbiotec.2020.04.013>.
- Ekwealor, C. C., John, F., Odibo, C., & Onwosi, C. O. (2017). Partial Purification and Characterization of Cellulase Produced by *Bacillus sphaericus* CE-3. *Advances in Microbiology*, 7, 293–303. <https://doi.org/10.4236/aim.2017.74024>.
- Ezeilo, U. R., Lee, C. T., Huyop, F., Zakaria, I. I., & Wahab, R. A. (2019). Raw oil palm frond leaves as cost-effective substrate for cellulase and xylanase productions by *Trichoderma asperellum* UC1 under solid-state fermentation. *Journal of Environmental Management*, 243, 206–217. <https://doi.org/10.1016/j.jenvman.2019.04.113>.
- Ezeilo, U. R., Wahab, R. A., & Mahat, N. A. (2020). Optimization studies on cellulase and xylanase production by *Rhizopus oryzae* UC2 using raw oil palm frond leaves as substrate under solid state fermentation. *Renewable Energy*, 156, 1301–1312. <https://doi.org/10.1016/j.renene.2019.11.149>.
- Fagbohunka, B. S., Okonji, R. E., & Adenike, A. Z. (2017). Purification and Characterization of Cellulase from Termite *Ametermes eveuncifer* (Silverstri) Soldiers. *Internasional Journal of Biology*, 9(1), 1–9. <https://doi.org/10.5539/ijb.v9n1p1>.
- Fathollahi, Z., Habibpour, B., Imani, S., Hassanzadeh, N., & Abdigoudarzi, M. (2021). Identification of Cellulolytic Bacteria from Guts of *Microcerotermes diversus* Silvestri (Isoptera: Termitidae) in Southern Iran. *Current Microbiology*, 78(4), 1305–1316. <https://doi.org/10.1007/s00284-021-02369-0>.
- Feofilova, E. P., & Mysyakina, I. S. (2016). Lignin: Chemical structure, biodegradation, and practical application (a review). *Applied Biochemistry and Microbiology*, 52(6), 573–581. <https://doi.org/10.1134/S0003683816060053>.
- Ferbiyanto, A., Rusmana, I., & Raffiudin, R. (2015). Characterization and Identification of Cellulolytic Bacteria from gut of Worker *Macrotermes gilvus*. *Hayati Journal of Biosciences*, 22(4), 197–200. <https://doi.org/10.1016/j.hjb.2015.07.001>.
- Gergonius, F., & Sine, Y. (2016). Isolasi Dan Uji Biokimia Bakteri Selulolitik Asal Saluran Pencernaan Rayap Pekerja (*Macrotermes* spp.). *Bio-Edu: Jurnal Pendidikan Biologi*, 1(2), 27–29. <https://jurnal.unimor.ac.id/JBE/article/view/501>
- Gupta, R., Gigras, P., Mohapatra, H., Goswami, V.K., Chauhan, B., (2003). Microbial α -amylases: a biotechnological perspective. *Process Biochem.* 38, 1599–1616.
- Handayani, W., & Aji, W. (2020). Keanekaragaman Makrofauna Tanah Pada Beberapa Penggunaan Lahan Gambut. *Jurnal Agroforestri Indonesia*, 3(2), 77–88.
- Hermansyah, Fachrijal, Miksusanti, Fatma, Kasmiarti, G., & Panagan, A. T. (2019). Xylose and arabinose fermentation to produce ethanol by isolated yeasts from durian (*Durio zibethinus*) fruit. *Molekul*, 14(2), 133–139. <https://doi.org/10.20884/1.jm.2019.14.2.562>.
- Hermansyah, Novia, & Wiraningsih, M. (2016). Bioethanol Production From Cellulose by *Candida tropicalis*, as An Alternative Microbial Agent to Produce Ethanol from Lignocellulosic Biomass. *Sriwijaya Journal of Environment*, 1(1), 10–13.

- <https://doi.org/10.22135/sje.2016.1.1.10-13>.
- Hermansyah, Sakinah, R. A., Julinar, J., Hanafiah, Z., & Zulkifli, H. (2018). Bioethanol Production from *Microalgae Oscillatoria* sp Cultured in Blue Green 11 and Bold Basal Media. *E3S Web of Conferences*, 68, 4–11. <https://doi.org/10.1051/e3sconf/20186803018>.
- Hermansyah, Xayasene, T., Huu Tho, N., Miksusanti, M., Fatma, F., & Panagan, A. T. (2018). Bioethanol Production from Cassava (*Manihot esculenta*) Peel Using Yeast Isolated from Durian (*Durio zhibetinus*). *Journal of Physics: Conference Series*, 1095(1). 1–7. <https://doi.org/10.1088/1742-6596/1095/1/012016>.
- Hidayat, M. R. (2021). Isolation and identification of cellulolytic bacteria symbiont from various termites on different nest type in bukit baka bukit raya national park, West Kalimantan, Indonesia. *Walailak Journal of Science and Technology*, 18(14). <https://doi.org/10.48048/wjst.2021.12708>.
- Hongoh, Y. (2011). Toward the functional analysis of uncultivable, symbiotic microorganisms in the termite gut. *Cellular and Molecular Life Sciences*, 68(8), 1311–1325. <https://doi.org/10.1007/s00018-011-0648-z>.
- Hou, R., Hu, J., Wang, Y., Wei, H., & Gao, M. (2019). Simultaneous production of cellulase and ferulic acid esterase by *Penicillium decumbens* with rice straw as the sole carbon source. *Journal of Bioscience and Bioengineering*, 129(3). <https://doi.org/10.1016/j.jbiosc.2019.09.013>.
- Husseneder, C., Billy, R., Wise, Dennis, T., Higashiguchi. (2005). Microbial Diversity In The Termite Gut: A Complementary Approach Combining Culture And Culture-Independent Techniques. (pp. 189–195). *Proceedings Of The Fifth International Conference On Urban Pests*.
- Ilmen, M., Saloheimo, A., Onnela, M. L., Penttila, M. E. (1997). Regulation of cellulase gene expression in the filamentous fungus *Trichoderma reesei*. *Appl Environ Microbiol.* 63, 1298–1306.
- Iqbal, H. M. N., Kyazze, G., & Keshavarz, T. (2013). Advances in the valorization of lignocellulosic materials by biotechnology: An overview. *BioResources*, 8(2), 3157–3176. <https://doi.org/10.15376/biores.8.2.3157-3176>.
- Jalaludin, N.-A., Rahim, F., & Yaakop, S. (2018). Termite Associated to Oil Palm Stands in Three Types of Soils. *Sains Malaysiana*, 47(9), 1961–1967.
- Javaheri-Kermani, M., & Asoodeh, A. (2019). A novel beta-1,4 glucanase produced by symbiotic *Bacillus* sp. CF96 isolated from termite (*Anacanthotermes*). *International Journal of Biological Macromolecules*, 131, 752–759. <https://doi.org/10.1016/j.ijbimac.2019.03.124>.
- Karthika, A., Seenivasagan, R., Kasimani, R., Babalola, O. O., & Vasanthy, M. (2020). Cellulolytic bacteria isolation , screening and optimization of enzyme production from vermicompost of paper cup waste. *Waste Management*, 116, 58–65. <https://doi.org/10.1016/j.wasman.2020.06.036>.
- Keshk, S. M. A. S. (2016). Cellulase Application in Enzymatic Hydrolysis of Biomass. In *New and Future Developments in Microbial Biotechnology and Bioengineering: Microbial Cellulase System Properties and Applications*. Elsevier B.V. <https://doi.org/10.1016/B978-0-444-63507-5.00016-2>.
- Kumar, B., & Verma, P. (2020). Enzyme mediated multi-product process: A concept

- of bio-based refinery. *Industrial Crops and Products*, 154, 112607. <https://doi.org/10.1016/j.indcrop.2020.112607>.
- Kus, J. V. (2014). Infections due to *Citrobacter* and *Enterobacter*. In *Reference Module in Biomedical Sciences* (Issue August). Elsevier Inc. <https://doi.org/10.1016/b978-0-12-801238-3.05089-3>.
- Li, C., Li, D., Feng, J., Fan, X., Chen, S., Zhang, D., & He, R. (2019). Duckweed (*Lemna minor*) is a novel natural inducer of cellulase production in *Trichoderma reesei*. *Journal of Bioscience and Bioengineering*, 127(4), 486–491. <https://doi.org/10.1016/j.jbiosc.2018.09.017>.
- Li, Q., Ng, W. T., & Wu, J. C. (2014). Isolation, characterization and application of a cellulose-degrading strain *Neurospora crassa* S1 from oil palm empty fruit bunch. *Microbial Cell Factories*, 13(1), 1–8. <https://doi.org/10.1186/s12934-014-0157-5>.
- Libardi, N., Soccol, C. R., Góes-Neto, A., Oliveira, J. de, & Vandenberghe, L. P. de S. (2017). Domestic wastewater as substrate for cellulase production by *Trichoderma harzianum*. *Process Biochemistry*, 57, 190–199. <https://doi.org/10.1016/j.procbio.2017.03.006>.
- Lin, Y., & Tanaka, S. (2006). Ethanol fermentation from biomass resources: Current state and prospects. *Applied Microbiology and Biotechnology*, 69(6), 627–642. <https://doi.org/10.1007/s00253-005-0229-x>.
- Lodha, A., Pawar, S., & Rathod, V. (2020). Optimised cellulase production from fungal co-culture of *Trichoderma reesei* NCIM 1186 and *Penicillium citrinum* NCIM 768 under solid state fermentation. *Journal of Environmental Chemical Engineering*, 8(5), 103958. <https://doi.org/10.1016/j.jece.2020.103958>.
- Makherjee, S. (2019). Isolation and Purification of Industrial Enzymes: Advances in Enzyme Technology. In *Biomass, Biofuels, Biochemicals, Advances in Enzyme Technology*. Elsevier B. V. <https://doi.org/10.1016/B978-0-444-64114-4.00002-9>.
- Mrudula, S., Murugammal, R., (2011). Production of cellulase by *Aspergillus niger* under submerged and solid-state fermentation using coir waste as a substrate. *Braz. J. Microbiol.* 42(3), 1119–1127.
- Mulyani, P. D., Hamid, R. M., Janatunaim, R. Z., & Purwestri, Y. A. (2018). Amylolytic ability of bacteria isolated from termite (*Coptotermes* sp.) gut. *Indonesian Journal of Biotechnology*. 23(1), 14–20. <https://doi.org/10.22146/ij-biotech.32445>.
- Nair, A. S., Al-Battashi, H., Al-Akzawi, A., Annamalai, N., Gujarathi, A., Al-Bahry, S., Dhillon, G. S., & Sivakumar, N. (2018). Waste office paper: A potential feedstock for cellulase production by a novel strain *Bacillus velezensis* ASN1. *Waste Management*, 79, 491–500. <https://doi.org/10.1016/j.wasman.2018.08.014>.
- Navaneethapandian, U., Kumar, A. G., Liduja, K., & Jayachithra, R. (2021). Biocatalyst : Cellulase Production in Solid State Fermentation (SSF) Using Rice Bran as Substrate. *Biointerface Research in Applied Chemistry*. 11(1), 7689–7699.
- Neoh, K. B., Bong, L. J., Muhammad, A., Itoh, M., Kozan, O., Takematsu, Y., & Yoshimura, T. (2017). The effect of remnant forest on insect successional response in tropical fire impacted peatland: A Bi-Taxa comparison. *PLoS ONE*,

- 12(3), 1–17. <https://doi.org/10.1371/journal.pone.0174388>.
- Ngangi, J., Pelealu, J., Warouw, J., & Mandey, L. (2013). Isolation and Activity of Cellulolytic Bacteria Isolated from Hindgut of *Odontotermes* spa Subteran Termite On Wasian (*Elmerrelia celebica* L.) an Endemic Wood to North Sulawesi. *International Journal of Science and Engineering Investigations*. 2(22), 8–16.
- Ni, J., & Tokuda, G. (2013). Lignocellulose-degrading enzymes from termites and their symbiotic microbiota. *Biotechnology Advances*, 31(6), 838–850. <https://doi.org/10.1016/j.biotechadv.2013.04.005>.
- Pabbathi, N. P. P., Velidandi, A., Tavarana, T., Gupta, S., Raj, R. S., Gandam, P. K., & Baadhe, R. R. (2021). Role of metagenomics in prospecting novel endoglucanases, accentuating functional metagenomics approach in second-generation biofuel production: a review. *Biomass Conversion and Biorefinery*. 7, 1–28. <https://doi.org/10.1007/s13399-020-01186-y>.
- Pandey, A. K., & Negi, S. (2020). Biocatalysis and Agricultural Biotechnology Enhanced cellulase recovery in SSF from *Rhizopus oryzae* SN5 and immobilization for multi - batch saccharification of carboxymethylcellulose. *Biocatalysis and Agricultural Biotechnology*, 26, 1–7. 101656. <https://doi.org/10.1016/j.bcab.2020.101656>.
- Pandiyani, K., Tiwari, R., Singh, S., Nain, P. K. S., Rana, S., Arora, A., Singh, S. B., & Nain, L. (2014). Optimization of Enzymatic Saccharification of Alkali Pretreated *Parthenium* sp . Using Response Surface Methodology. *Hindawi Publishing Corporation Enzyme*. 2014, 1–8.
- Pang, J., Wang, J., Liu, Z., Zhang, Q., & Qi, Q. (2019). Identification and characterization of an Endo-glucanase secreted from cellulolytic *Escherichia coli* ZH-4. *BMC Biotechnology*, 19(1), 1–9. <https://doi.org/10.1186/s12896-019-0556-0>.
- Pelczar, M. J., Chan, E. C. S., and Krieng, N. R. (2005). *Microbiology* (5th Ed. 793-811), Tata McGraw Hill, New Delhi.
- Peristiwati, Natamihardja, Y. S., & Herlini, H. (2018). Isolation and identification of cellulolytic bacteria from termites gut (*Cryptotermes* sp.). *Journal of Physics: Conference Series*, 1013(1). <https://doi.org/10.1088/1742-6596/1013/1/012173>.
- Ramin, M., Alimon, A. R., Abdullah, N., Panandam, J. M., & Sijam, K. (2008). Isolation and identification of three species of bacteria from the termite *Coptotermes curvignathus* (Holmgren) present in the vicinity of University Putra Malaysia. In *Research Journal of Microbiology* (Vol. 3, Issue 4, pp. 288–292). Academic Journals Inc. <https://doi.org/10.3923/jm.2008.288.292>.
- Saini, A., Aggarwal, N. K., & Yadav, A. (2017). Cost-effective cellulase production using *Parthenium hysterophorus* biomass as an unconventional lignocellulosic substrate. *3 Biotech*, 7(1), 1–11. <https://doi.org/10.1007/s13205-017-0604-1>.
- Scopes, R. K. (2002). Enzyme Activity and Assays. *Encyclopedia of Life Sciences*. MacMillan Publishers Ltd. Nature Publishing Group. 1–6. <https://doi.org/10.1038/npg.cls.0000712>.
- Shard, A. G., Schofield, R. C., & Minelli, C. (2019). Ultraviolet-visible

- spectrophotometry. In *Characterization of Nanoparticles: Measurement Processes for Nanoparticles* (Vol. 1). Elsevier Inc. <https://doi.org/10.1016/B978-0-12-814182-3.00012-2>.
- Sharma, A., & Tewari, R. (2016). Cellulases: Classification, Methods of Determination and Industrial Applications. *Applied Biochemistry and Biotechnology*, 179, 1346–1380. <https://doi.org/10.1007/s12010-016-2070-3>.
- Sharma, D., Joshi, B., Bhatt, M. R., Joshi, J., Malla, R., & Bhattarai, T. (2015). Isolation of Cellulolytic Organisms from the Gut Contents of Termites Native to Nepal and Their Utility in Saccharification and Fermentation of Lignocellulosic Biomass. *Journal of Biomass to Biofuel*, 2, 11–20. <https://doi.org/10.11159/jbb.2015.002>.
- Shen, C.-H. (2019). Characterization of Nucleic Acids and Proteins. *Diagnostic Molecular Biology, DNase I*, 249–276. <https://doi.org/10.1016/b978-0-12-802823-0.00010-9>.
- Show, B. K., Banerjee, S., Banerjee, A., Thakur, R. G., Hazra, A. K., Mandal, N. C., Ross, A. B., Balachandran, S., Chaudhury, S. (2022). Insect gut bacteria: a promising tool for enhanced biogas production. *Rev Environ Sci Biotechnol*, 21, 1–25. <https://doi.org/10.1007/s11157-021-09607-8>.
- Sil, S., Kumar De, K., & Ghosh, A. (2021). Phylogenetic analysis of six different species of *Saraca* L. (fabaceae, caesalpinioideae) based on chloroplast matk gene. *Biodiversitas*, 22(9), 3880–3889. <https://doi.org/10.13057/biodiv/d220934>.
- Silva, A. F. V., Santos, L. A., Valença, R. B., Porto, T. S., Da Motta Sobrinho, M. A., Gomes, G. J. C., Jucá, J. F. T., & Santos, A. F. M. S. (2019). Cellulase production to obtain biogas from passion fruit (*Passiflora edulis*) peel waste hydrolysate. *Journal of Environmental Chemical Engineering*, 7(6), 1–8. 103510. <https://doi.org/10.1016/j.jece.2019.103510>.
- Sinha, D., Banerjee, S., Mandal, S., Basu, A., Banerjee, A., Balachandran, S., Chandra Mandal, N., & Chaudhury, S. (2021). Enhanced biogas production from *Lantana camara* via bioaugmentation of cellulolytic bacteria. *Bioresource Technology*, 340, 1–10. <https://doi.org/10.1016/j.biortech.2021.125652>.
- Sreeremya, S., Nishaa, S., & Rajiv, P. (2016). Optimization of Conditions and Production of Carboxy Methyl Cellulase by Bacteria Isolated from Higher Termite Soil. *Journal of Bioprocessing & Biotechniques*, 6(2), 6–9. <https://doi.org/10.4172/2155-9821.1000270>.
- Stepnov, A. A., Fredriksen, L., Steen, I. H., Stokke, R., & Eijsink, V. G. H. (2019). Identification and characterization of a hyperthermophilic GH9 cellulase from the Arctic Mid-Ocean Ridge vent field. *PLoS ONE*, 14(9), 1–19. <https://doi.org/10.1371/journal.pone.0222216>.
- Sukumaran, R. K., Singhania, R. R., Mathew, G. M., & Pandey, A. (2009). Cellulase production using biomass feed stock and its application in lignocellulose saccharification for bio-ethanol production. *Renewable Energy*, 34(2), 421–424. <https://doi.org/10.1016/j.renene.2008.05.008>.
- Susilowati D., Subekti, N. and Bintari, S. H. (2018). The Potential of Microbial Symbiosis *Macrotermes gilvus* Hagen Termite Gut as Degrading Agents of Cellulose in Bioethanol Production. *Biosaintifika*, 10(2), 395–400.
- Swathy, R., Rambabu, K., Banat, F., Ho, S. H., Chu, D. T., & Show, P. L. (2020).

- Production and optimization of high grade cellulase from waste date seeds by *Cellulomonas uda* NCIM 2353 for biohydrogen production. *International Journal of Hydrogen Energy*, 45(42), 22260–22270. <https://doi.org/10.1016/j.ijhydene.2019.06.171>.
- Teter, S. A., Sutton, K. B., Emme, B., (2014). Enzymatic processes and enzyme development in biorefining. (133-299). Woodhead Publishing Limited, USA. <https://doi.org/10.1533/9780857097385.1.199>.
- Thao, T. P., Minh Duc, N., Hien, V. T., & Lanh, D. T. (2012). Evaluation of the Effects of *Metarhizium* (M1) for the Control of Subterranean Termite *Coptotermes Formosanus*, Proceedings of the 9th Pacific-Rim Termite Research Group Conference. *February*, 141–146. <http://www.prtrg.org/images/proceedingsTRG-9.pdf#page=141>.
- Titilayo, F., Ekiti, A., Adebayo, A. A., & Ekiti, A. (2022). Production And Partial Purification Of Extracellular Cellulase By *Bacillus Cereus* Isolated From Agricultural Waste In Ado-Ekiti Production And Partial Purification Of Extracellular Cellulase By *Bacillus Cereus* Isolated From Agricultural Waste In Ado-Ekiti. *Global Scientific Journal*. 9(3), 1743–1757.
- Vallinachiyar, S. A. J. C. (2011). Optimization of cellulase production by *Aspergillus nidulans*: application in the biosoftening of cotton fibers. *World Journal of Microbiology and Biotechnology*. 27, 85–97. <https://doi.org/10.1007/s11274-010-0431-6>.
- Varghese, L. M., Agrawal, S., Sharma, D., Mandhan, R. P., & Mahajan, R. (2017). Cost-effective screening and isolation of xylano-cellulolytic positive microbes from termite gut and termitarium. *3 Biotech*, 7(2), 1–7. <https://doi.org/10.1007/s13205-017-0733-6>.
- Varma, Y., Chovatiya, S., Patel, D., Ingle, S., Daxini, N., & Lal, S. (2021). Media Optimization And Saccharification of Lignocellulosic Waste By Cellulolytic Bacteria Isolated From Gut of A Termite. *Journal of Biotechnology Trends and Technology*. 11(2), 8–14 <https://doi.org/10.14445/22490183/IJBTT-V11I2P602>.
- Verma, N., Kumar, V., & Bansal, M. C. (2018). Biocatalysis and Agricultural Biotechnology Utility of *Luffa cylindrica* and *Litchi chinensis* peel , an agricultural waste biomass in cellulase production by *Trichoderma reesei* under solid state cultivation. *Biocatalysis and Agricultural Biotechnology*, 16, 483–492. <https://doi.org/10.1016/j.bcab.2018.09.021>.
- Vilanova, C., Marco, G., Domínguez-Escribà, L., Genovés, S., Sentandreu, V., Bataller, E., Ramón, D., & Porcar, M. (2012). Bacteria from acidic to strongly alkaline insect midguts: Potential sources of extreme cellulolytic enzymes. *Biomass and Bioenergy*, 45, 288–294. <https://doi.org/10.1016/j.biombioe.2012.06.017>.
- Walker, J. M. (2002). *The Protein Protocols Handbook* (2nd Ed.) Humana Press, Totowa, New Jersey.
- Wright, J. P., & Jones, C. G. (2006). The concept of organisms as ecosystem engineers ten years on: Progress, limitations, and challenges. *BioScience*, 56(3), 203–209. [https://doi.org/10.1641/0006-3568\(2006\)056\[0203:TCCOAE\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2006)056[0203:TCCOAE]2.0.CO;2).

- Wu, C. C., Tsai, C. L., Liang, W. R., Takematsu, Y., & Li, H. F. (2019). Identification of Subterranean Termite Genus, *Reticulitermes* (Blattodea: Rhinotermitidae) in Taiwan. *Journal of Economic Entomology*, 112(6), 2872–2881. <https://doi.org/10.1093/jee/toz183>.
- Xue, D., Yao, D., Sukumaran, R. K., You, X., Wei, Z., & Gong, C. (2020). Tandem integration of aerobic fungal cellulase production, lignocellulose substrate saccharification and anaerobic ethanol fermentation by a modified gas lift bioreactor. *Bioresource Technology*, 302, 122902. <https://doi.org/10.1016/j.biotech.2020.122902>.
- Yadav, A. N., Gupta, A., Mishra, S., & Singh, S. (2019). Recent Advancement in White Biotechnology Through Fungi: *Perspective for Sustainable Environments* (Vol. 3, 303–361). Springer. <https://doi.org/10.1007/978-3-030-10480-1>.
- Yong Syuan, K., Ong Gaik Ai, L., & Kim Suan, T. (2018). Evaluation of cellulase and xylanase production from *Trichoderma harzianum* using acid-treated rice straw as solid substrate. *Materials Today: Proceedings*, 5(10), 22109–22117. <https://doi.org/10.1016/j.matpr.2018.07.077>.
- Zhao, C., Xie, B., Zhao, R., & Fang, H. (2019). Microbial oil production by *Mortierella isabellina* from sodium hydroxide pretreated rice straw degraded by three-stage enzymatic hydrolysis in the context of on-site cellulase production. *Renewable Energy*, 130, 281–289. <https://doi.org/10.1016/j.renene.2018.06.080>.