

Review

Termite Microbial Symbiosis as a Model for Innovative Design of Lignocellulosic Future Biorefinery: Current Paradigms and Future Perspectives

Mudasir A. Dar ^{1,*}, Rongrong Xie ^{1,†}, Hossain M. Zayed ², Shehbaz Ali ¹, Daochen Zhu ¹ and Jianzhong Sun ^{1,*}

¹ Biofuels Institute, School of Environment and Safety Engineering, Jiangsu University, Zhenjiang 212013, China; rxie@ujs.edu.cn (R.X.); shehbaz@ujs.edu.cn (S.A.); dczhu@ujs.edu.cn (D.Z.)

² School of Life Science, Guangzhou University, 230 Wai Huan Xi Road, Guangzhou 510006, China; zayed@gzhu.edu.cn

* Correspondence: muddar7@ujs.edu.cn (M.A.D.); jzsun1002@ujs.edu.cn (J.S.); Tel.: +86-15052919625 (J.S.); Fax: +86-0511-88790955 (J.S.)

† These authors contributed equally to this work.

Abstract: The hunt for renewable and alternative fuels has driven research towards the biological conversion of lignocellulosic biomass (LCB) into biofuels, including bioethanol and biohydrogen. Among the natural biomass utilization systems (NBUS), termites represent a unique and easy-to-access model system to study host–microbe interactions towards lignocellulose bioconversion/valorization. Termites have gained significant interest due to their highly efficient lignocellulolytic systems. The wood-feeding termites apply a unique and stepwise process for the hydrolysis of lignin, hemicellulose, and cellulose via biocatalytic processes; therefore, mimicking their digestive metabolism and physiochemical gut environments might lay the foundation for an innovative design of nature-inspired biotechnology. This review highlights the gut system of termites, particularly the wood-feeding species, as a unique model for future biorefinery. The gut system of termites is a treasure-trove for prospecting novel microbial species, including protists, bacteria, and fungi, having higher biocatalytic efficiencies and biotechnological potentials. The significance of potential bacteria and fungi for harnessing the enzymes appropriate for lignocellulosic biorefinery is also discussed. Termite digestomes are rich sources of lignocellulases and related enzymes that could be utilized in various industrial processes and biomass-related applications. Consideration of the host and symbiont as a single functioning unit will be one of the most crucial strategies to expedite developments in termite-modeled biotechnology in the future.

Keywords: lignocellulose biorefinery; termites; gut system; gut microbiota; lignocellulose valorization; biomimetics



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1. Introduction

The dwindling of fossil fuels together with increasing greenhouse gas emissions are the key challenges to modern societies. The exploration of novel and renewable energy resources is imperative for sustainable development. Lignocellulose comprises one such renewable and abundant energy resource that can be harvested throughout the year [1]. Each year, lignocellulose is produced in vast quantities through photosynthesis. The LCB includes municipal solid waste (MSW), specialized agricultural and forestry residues, and dedicated energy crops that are abundantly available and possess the necessary attributes for the reduction of greenhouse gas emissions. The biological hydrolysis mediated through the enzymes is considered a valuable approach for lignocellulose valorization, as it provides pure and sustainable products [2]. In addition, noteworthy advancements in technology and protein engineering strategies, such as system biology and immobilization, have

been achieved in recent years to enhance enzyme properties as well as overall catalytic efficiencies for a higher yield of products [3]. Through enzymatic hydrolysis mediated by cellulases and hemicellulases, lignocellulose can be successfully transformed into simple sugars, which can undergo fermentation to produce ethanol and other sources of energy. This could prove an effective and practical method for the production of novel bioenergy sources [4,5]. For instance, biofuels can be generated from the lignocellulose via enzymatic bioconversion and fermentation [6]. The annual production of LCB is 3–5 GT worldwide, which could supply about 50–85 EJ of energy per year, which accounts for 10–20% of the world's current energy demands [7]. Besides the bioenergy production from LCB, it results in the net saving of energy and reduced industrial CO₂ emissions while fixing CO₂ in the soil with perennial energy crops [8]. The bioconversion of lignocellulose into energy and commodity chemicals is more sensible; however, its hydrolysis into chemicals seems plausible and more efficient to make use of its mass and atoms [9]. Therefore, the bioconversion of lignocellulose into energy materials and value-added chemicals is critical for sustainable development while supplying renewable energy and safeguarding the environment.

In nature, LCB is consumed and utilized by various natural biomass utilization systems (NBUS), such as insects, termites, snails, rumen, etc. [10–12]. These organisms possess tremendous success in utilizing LCB as their main food. However, among these NBUS, termites represent the most advanced and highly efficient lignocellulolytic machineries [4]. These sophisticated lignocellulolytic systems are attributed to symbiotic associations with gut microbes, such as bacteria, protists, and fungi [13]. Considering their dietary reliance on complex lignocellulosic materials, termites harbor a militia of microbiota in the gut systems that exhibit proficiency in the production of lignocellulose-degrading enzymes [14]. Owing to the technological advancements, a wealth of information has emerged in the past decades on lignocellulose decomposition by termites and their potential applications in biorefinery [15]. To this end, herein, we have discussed the practical implications of the termite gut systems, particularly lignocellulolytic systems derived from microbial symbionts. Further, we have elaborated on the potential role of the termite systems as a unique model for future lignocellulosic biorefinery based on biomimetic strategies.

2. Bibliometric Analysis

For this study, a literature survey was performed in the Scopus abstracts and citations database (<http://www.scopus.com/>, accessed on 7 October 2023). This database is a well-known data source and preferred for bibliometric analysis. Further, the Scopus database is easily available and accessible across most universities' and research organizations' online library systems [16]. The terminology used included "Termite" plus "Lignocellulose" plus "Biofuels" as key terms. The search period ranged from 1950 afterwards (Figure 1). The total number of relevant papers were found to be 2130, with 1932 research articles, 36 book chapters, 6 preprints, and 25 dissertations. We specified the search criteria by focusing on the relevant literature published in the journals of the biology domain (Figure 2). Figure 1 shows the evolution of the subject over the last one century based on the number of scholarly works published, highlighting the tremendous attention from the scientific community in the last decade. The most productive years to date are 2018 and 2021; each reported 103 publications, accumulating 1946 citations. The countries that have contributed the most to the subject are the USA with 433 publications, the People's Republic of China with 177, Japan 156, India with 117, and Germany with 116 articles published. Further, the keyword co-occurrence assessment was performed based on the keywords provided by authors (Figure 3). The assessment revealed a strong network of biofuels with termites, cellulase, cellulolytic bacteria, lignocellulose, and cellulose.

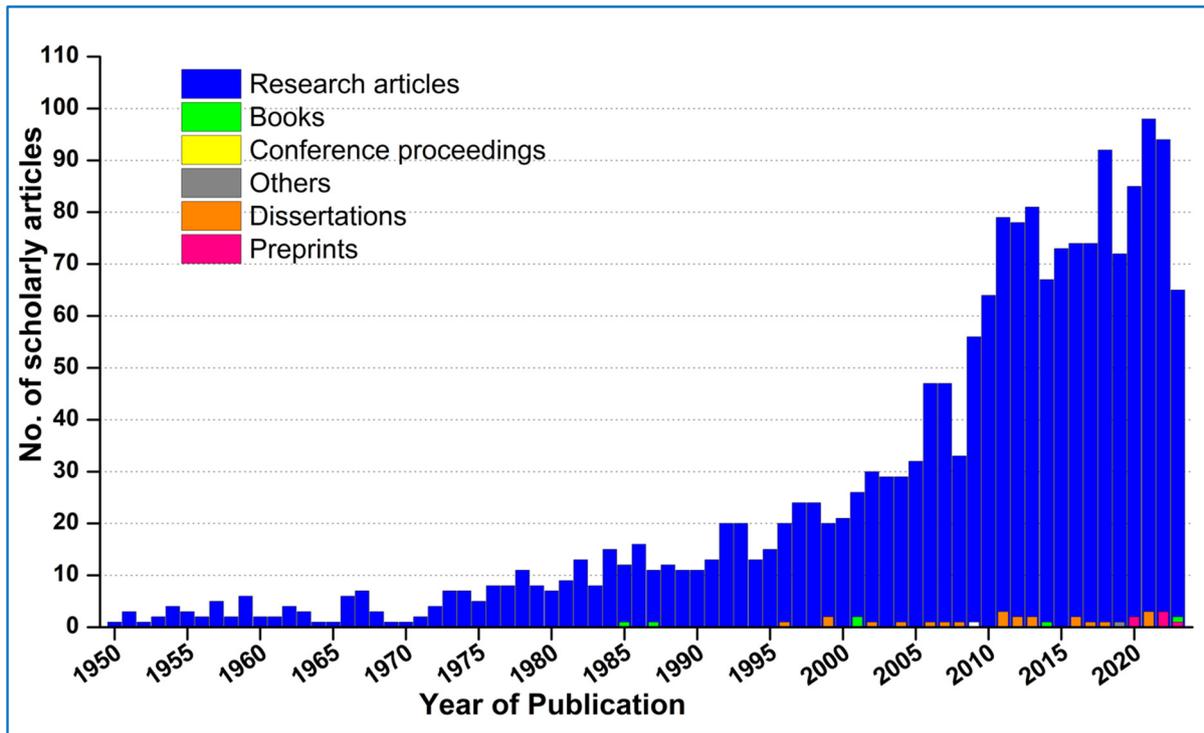


Figure 1. Total number of articles published between 1950 to 7 October 2023. The data were retrieved from the Scopus database by searching the key terminologies, such as termites, lignocellulosic biomass, and biofuel, in the title/abstract/author keywords of the published literature.

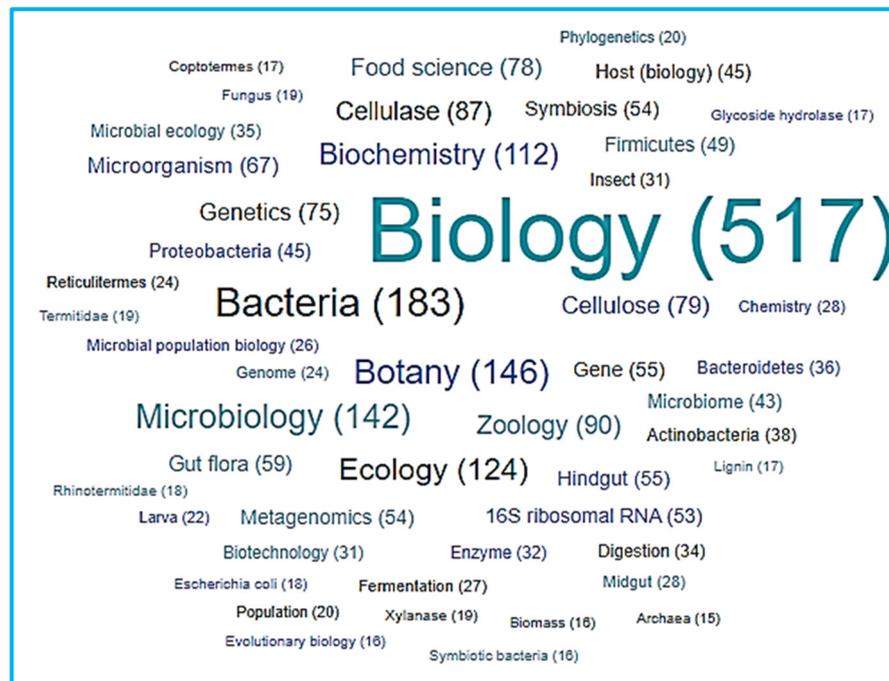


Figure 2. Word-cloud analysis of the termite gut bacteria representing the most frequent fields-of-study.

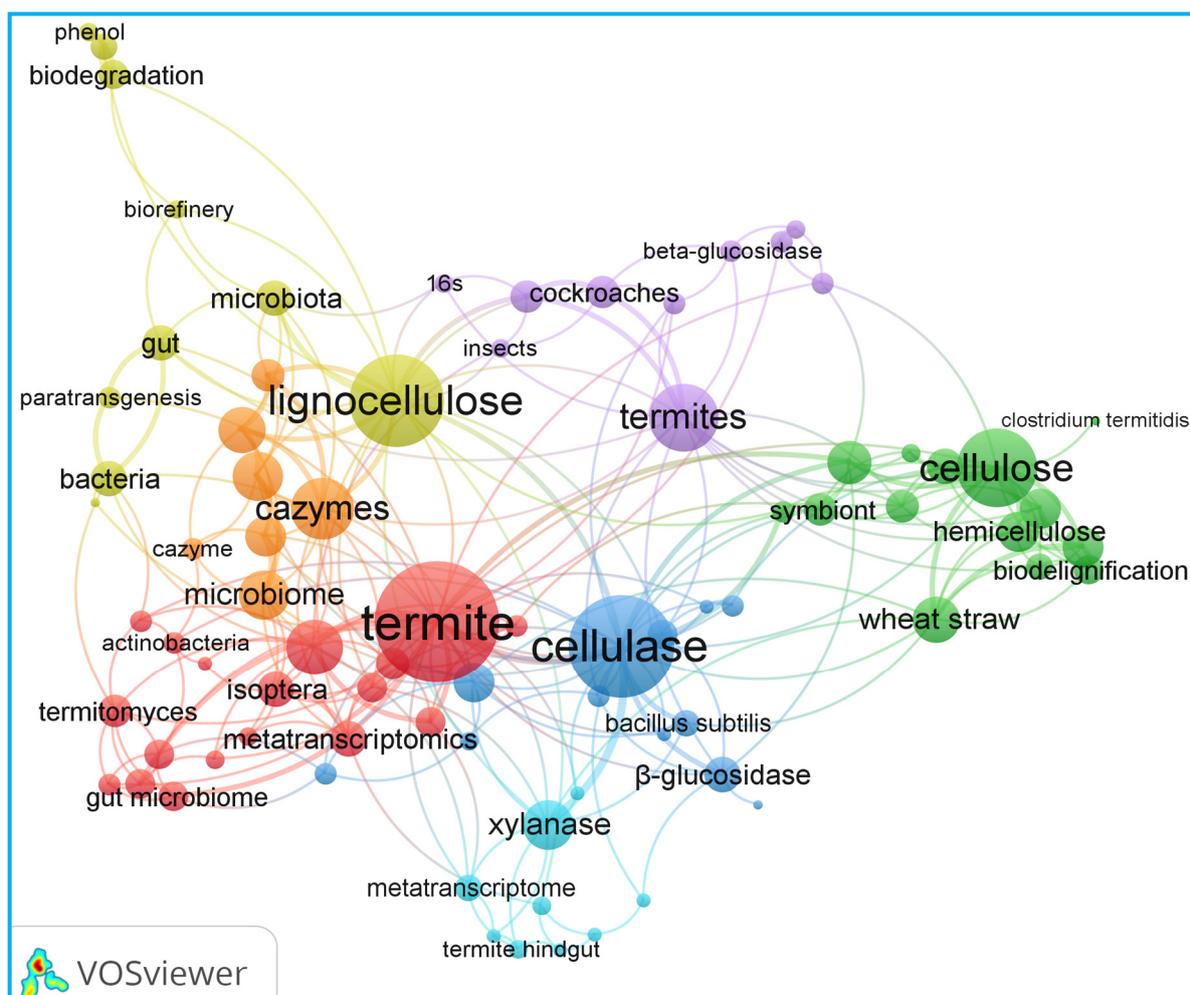


Figure 3. A keyword co-occurrence network analysis plotted by the VOSviewer software based on the bibliometric data retrieved from Scopus database.

3. Natural Lignocellulosic Biomass Utilization Systems

Lignocellulose is a sustainable and renewable carbon source found in most plants [17]. Researchers have shown interest in various kinds of plant biomass for the production of second-generation bioethanol. Agricultural residues, such as rice husk, corn cob, stover, wheat straw, and sugarcane bagasse, are being explored as promising sources of LCB. Recently, many studies have focused on forest-derived woody residues and biomass, including sawdust, bark, hardwood chips (oak), softwood trimmings (pine), coconut shell, poplar trees, or residues from gardens and parks (grasses, leaves, twigs) for bioethanol productions. The three major components of LCB include cellulose, hemicellulose, and lignin, among which the former two comprise about 70% of the plant biomass, while lignin constitutes about 25–35% depending upon the plant species and geography [18]. The composition of LCB is affected by the plant species, growth conditions, geography, developmental stage, etc. [19]. Due to the heterogeneous nature of lignocellulose, the enzymatic conversion involves several enzymes and cofactors chiefly laccases, cellulases, and xylanases [20]. Among them, cellulases and xylanases play a key role for the bioconversion and valorization of biomass into commodity chemicals, such as enzymes, amino acids, and sugars, as they hydrolyze cellulose and hemicellulose, respectively [21]. The key enzymes of this complex process include endo- β -1, 4-glucanases (also known as CMCase), exo- β -1, 4-glucanases (Avicellase), and β -1, 4-glucosidase (β -glucosidase) [17]. Endoglucanase acts on the β -1, 4 bonds within cellulose inner chains, cleaving large molecules into short stretches. Exoglucanases then target the short stretches liberating the terminal

disaccharide in the form of cellobiose. These cellobioses are finally broken by β -glucosidase into individual sugar residues, ready for absorption through diverse metabolic pathways. Similarly, xylanases are known to degrade complex hemicelluloses into fermentable sugars that can be harvested for biorefinery applications. Hitherto, several NBUS have been identified to secrete these enzymes in collaboration with gut symbionts, such as bacteria and fungi. In natural environments, a range of organisms, such as rumen, bacteria, fungi, insects, annelids, mollusks, and nematodes, have evolved remarkable abilities to utilize lignocellulose [22,23]. To accomplish this, these NBUS have developed sophisticated and highly specialized gut systems designed for the digestion of a variety of ingested foods, with a predominant focus on lignocellulose. These NBUS exhibit extra-ordinary efficiency in metabolizing various components of lignocellulose by secreting cellulose hydrolytic systems viz, cellulases [24,25]. Among the animals as NBUS, termites have developed an advanced hydrolysis strategy through collaboration with gut microbiota, particularly bacteria and fungi [26]. Hitherto, a tremendous variety of lignocellulose-degrading microbes has been isolated and studied from different termites [4].

4. Termites as Pests and Model Systems

Termites are small insects that dwell in diverse environments, such as soils, trees, and wooden structures. Termites are considered among the most successful insects, with wide distribution across all continents except Antarctica, though they mostly prefer tropical and subtropical regions [27]. Termites represent a unique social system, where the worker caste is blamed for disastrous damage to crops and timber [28]. Though not all species, about 10% of the total described termites act as pests of wooden structures, including trees [29]. Termites cause significant damage to timber and wooden buildings, causing the buckling of wood, swollen floors and ceilings, etc. In the USA alone, termites inflict economic damage totaling billions of dollars annually, and their impact on tropical agriculture results in economic costs amounting to millions of dollars (<https://www.orkin.com/termites/facts/statistics>, accessed on 10 October 2023). In some tropical countries, termites attack living trees, causing heavy damage to the forests, thereby affecting afforestation. The termites that damage forests include the species of *Odontotermes* and *Microtermes*, which are also known as fungus-growing termites. They commence their attack in the top 15–25 cm of the ground. Other termites attack wooden houses or fences. During droughts, termites can consume as much plant material as cattle and game animals combined, thus increasing competition for fodder and forage. Contrary to damage, termites also serve many ecosystem services, such as mineral recycling, enhance soil fertility and crop yields, etc. [30], due to which they are also called ecosystem engineers. Termites have been considered important detritivores for the decomposition of lignocellulose in tropical environments, savannas, and forests as well as desert environments. From the sustainability and renewable bioenergy viewpoints, termites are designated as efficient natural bioreactors on Earth due to their tremendous efficiency for lignocellulose bioconversion [31].

Termites are perhaps the most researched insects to date due to their multiple characteristics, such as host–microbe interactions, their pest nature, and nitrogen or carbon recycling. Termites consume lignocellulosic biomass as their main food, which is the most abundant carbohydrate polymer on the Earth. Among different castes of termites, the workers perform feeding responsibilities and digest tremendous amounts of lignocellulose. This LCB is ingested in the form of wood or sometimes as partially decomposed material [32]. However, its prompt degradation by termites has attracted the attention of the scientific community all over the world. Termites achieve this remarkable efficiency in lignocellulose utilization through symbiosis with microbes residing in their gut systems [27]. The symbiotic bioconversion of lignocellulose into useful sugars has garnered significant interest in recent years due to its industry-oriented applications, such as biorefinery. Based on the scientific research of over one century, termites represent a unique and easy-to-access model system to study symbiotic processes. Being rivals to mammalian systems in terms

of microbial complexity and their impact towards host physiology, termites can be used as models to study host–microbe interactions. In addition, their astounding role in carbon recycling and lignocellulose degradation makes them sophisticated models for applied research, particularly for biorefinery.

Over the millions of years of evolution, termites have developed sophisticated lignocellulolytic systems with astonishing mechanisms to deal with the recalcitrance of lignocellulose. Due to exceptional biodegradation efficiency for lignocellulose bioconversion, termites are designated as promising natural bioreactors on Earth that may represent important models for the renewability and sustainability of bioenergy [31]. The biodegradation of LCB by termites is largely attributed to their gut symbiotic microbiota, particularly bacteria [33]. The gut system of wood-feeding termites may offer distinctive and novel models for engineering nature-inspired technologies with potential industrial applications. Since the onset of the twentieth century, many scientists have focused on termites as potential reservoirs of microorganisms and promising biocatalysts for the production of biofuels from renewable biomass [34].

5. Digestive System of Termites

Termite guts, especially of the worker caste, is very complicated, a coiled tube having a three-dimensional structure [35]. The gut is divisible into three major regions viz., foregut, midgut, and hindgut (Figure 4). The gut system of termites is a tube-like structure responsible for endogenous secretions, causing the primary digestion of ingested food. In the gut system, the lignin content of the biomass is partially hydrolyzed by the endogenous enzymes of the host. The mouth along with the anterior region of the gut plays a role in the mechanical grinding of ingested wood, while the posterior gut is involved in nutrient absorption and assimilation [36]. Though the fundamental plan remains common, the gut structure of lower termites varies slightly from higher termites. In the case of higher termites, the midgut is also known to secrete some endogenous cellulases. The gut in lower termites is relatively short and is slightly acidic, ranging between pH 5.5 to 7.0 [34]. As compared to lower termites, the hindgut of higher termites is more developed and complex in structure. Some species of higher termites have developed a further complex structure called a mixed segment (which is partially midgut and partially hindgut in origin) between the midgut and hindgut regions. In termites, the hindgut is the largest organ, which can be subdivided antero-posteriorly into multiple segments designated as P1, P2, P3, P4, and P5 [37]. Among these gut regions, P3 is the largest segment in higher termites. A marked difference between the soil-feeding and wood-feeding higher termites is the length of the P1 and P4 segments of the hindgut, where P1 is extended in wood feeders as compared to P4 in soil feeders [38]. The difference in physicochemical conditions in the gut regions affects the digestive physiology as well as symbiosis within the termites.

The hindgut region is a paunch-like bulbous region of actual digestion and the assimilation of LC biomass. It is also called the fermentation chamber due its functional role for the metabolism of cellulose into hydrogen and energy under anaerobic conditions by endosymbionts; therefore, it is the actual site that houses prokaryotes in all termites and also flagellates in the case of lower termites [39]. The gut of termites is an axially structured microhabitat exhibiting varying metabolic functions and microbial diversity [40]. Among the three gut sections, the relatively low diversity of microbiota is observed in the foregut and midgut as compared to the enlarged hindgut [27].

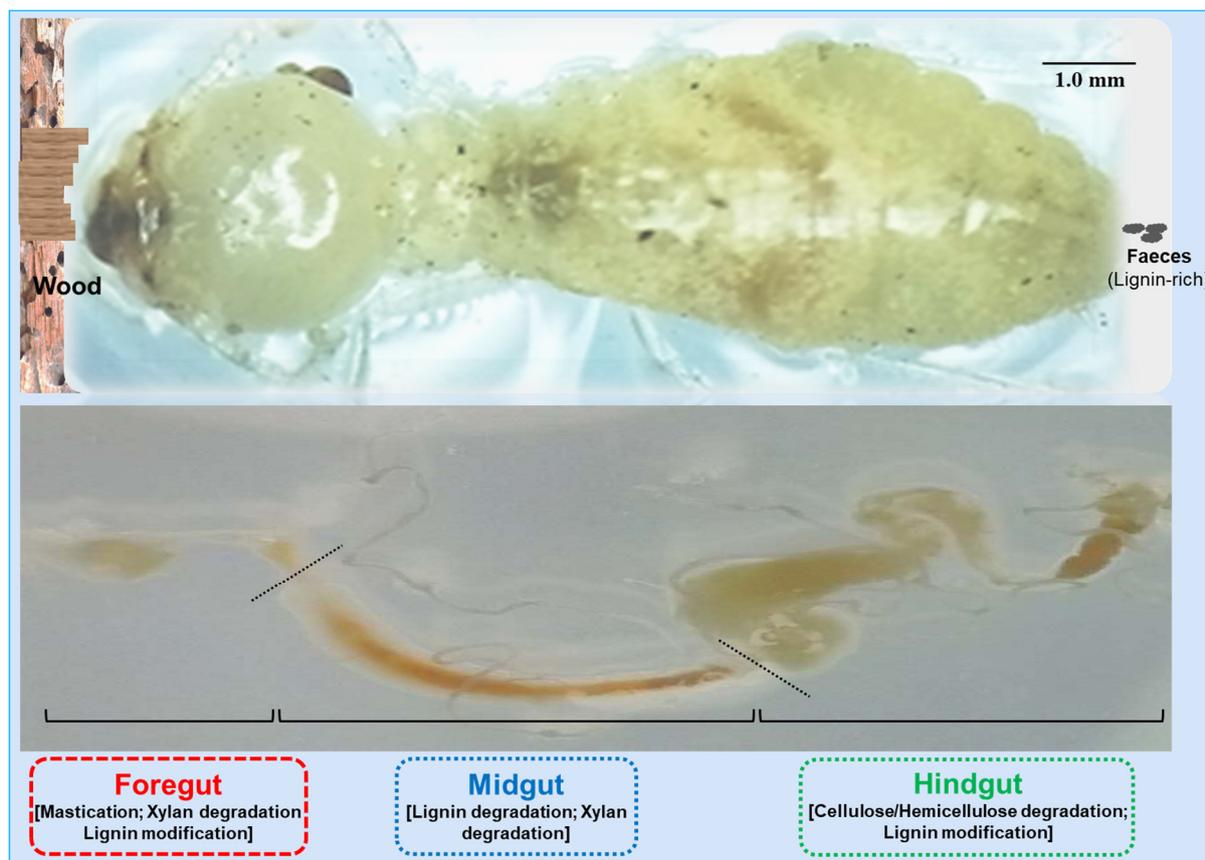


Figure 4. A typical example of the lower wood-feeding termite, *Coptotermes formosanus*, and its gut system showing the foregut, midgut, and hindgut regions.

6. Symbiosis of Termites with Gut Microbiota

Termite guts represent all three domains of life, such as Archaea, bacteria, eukaryotic protists, and fungi [4]. A plethora of information is available describing the synergism between the termite host, prokaryotes, and flagellates for lignocellulose hydrolysis. Additionally, the termite lignocellulolytic systems are unique among animals because they deconstruct lignin-derived compounds besides cellulosic plant materials. In fact, some researchers have estimated that the wood-feeding termite, *C. formosanus*, can produce 10^3 – 10^4 μmol of reducing sugar $\text{min}^{-1} \text{mL}^{-1}$ on carboxymethyl cellulose (CMC) as a substrate [27]. While genomic data have provided numerous evidences for the existence of cellulolytic activities in termites, it is imperative to complement such studies by isolating the corresponding proteins, conducting structural and functional analyses, and determining the localization of symbionts within the insect gut. Additionally, exploring their networks with cellulase complexes is crucial for a comprehensive understanding. Although the puzzle of termite–bacterial symbiosis is not resolved yet, it is near to resolution potentially due to technological advancements, such as molecular biology techniques. Molecular methods have revealed that the gut microbiota in termites carries out seven key activities, such as carbohydrate digestion, nitrogen fixation, nitrogen metabolism, oxygen consumption, hydrogen and methane productions, humification, deacetylation, and demethylation [29,41].

Termite–microbe symbiosis is perhaps the most well-known phenomenon that has been studied extensively in the recent past. The termite gut harbors a militia of microbiota from diverse taxa, such as bacteria, fungi, yeasts, flagellates, etc. [4]. In lower termites, such as *Coptotermes* and *Reticulitermes* species, flagellates are largely praised for their vital role in cellulose digestion, while endosymbiotic prokaryotes are known for acetogenesis, methanogenesis, nitrogen metabolism, and fermentation [36]. Over the past two decades, termite

biology has been researched significantly analyzing digestion, nitrogen metabolism, and biological symbiosis. Correspondingly, significant contributions have been accomplished to understand the structure of termite digestomes, their functions, and related physiologies. These enormous studies emphasize the need to understand termite–endosymbiont synergism to adopt an economic and sustainable technology for future biorefinery.

7. Termite Gut as Unique Reservoir of Lignocellulolytic Microorganisms

Termites share an intricate relationship with gut microbial symbionts, particularly for the digestion and assimilation of lignocellulose into energy and nutritional resources. The gut microbiota not only help the host to digest lignocellulose but also contribute enzymes and nutrients deficient in the hosts. Keeping in view the functions that symbionts contribute to the host, the gut microbiota in termites can be considered an integrated organ [42]. Cellulose being a macromolecule is cleaved by the bacterial enzymes into short-chain fatty acids in the gut system of termites [43]. These short chain fatty acids are further broken down and metabolized by the termites. Most of the nitrogen economy in termites is attributed to nitrogen fixation from symbiotic bacteria. Many other bacteria are reportedly involved in the synthesis of amino acids and production of cofactors [44–46].

Termites possess a battery of enzymes required for the degradation of LCB into fermentable products of hydrogen and energy. These enzymes are majorly contributed by the gut inhabitants involving flagellates, bacteria, and fungi. Not all termites contain flagellates, as higher termites are devoid of it, while bacteria are reserved by all termite species studied to date. Moreover, a tremendous diversity of bacteria has been represented in termite guts, reporting more than 200 species of bacterial genes. Termites achieve this lignocellulolytic expertise by collaborating with over 200 species of microbes that reside in their gut systems. The endosymbionts, such as “*Candidatus Endomicrobium trichonymphae*” and “*Candidatus Azobacteroides pseudotrichonymphae*”, that live within the unicellular flagellates produce their energy from the fermentation of carbohydrates to acetate [45,47]. These bacteria are substrate specific, where the former uses glucose-6-phosphate and later degrades glucose, xylose, or hexuronates only. It is likely due to the availability of these substrates within the termite gut system derived from the cellulose and hemicellulose digestion.

7.1. Termite Gut Bacteria

The termite gut system is a “gold mine” of symbiotic microorganisms, including bacteria, fungi, actinomycetes, and others. The total number of bacteria ranges from 10^7 – 10^{11} mL⁻¹ in the hindgut of termites [48]. Despite the small size of the termite gut, it offers a unique reservoir of novel microbes particularly bacteria that are found nowhere else in nature [49]. For the digestion of recalcitrant lignocellulose, wood-eating termites maintain a variety of unusual microbial symbionts, reaching densities of up to 10^{11} cells mL⁻¹ (Table 1). Termites shelter a diversity of lignocellulose-hydrolyzing bacteria that has been reported by many authors [4,27,38]. Bacteria belonging to four major phyla, such as Elusimicrobia, Bacteroidetes, Proteobacteria, and Actinobacteria, are found as endosymbionts in protist cells within the termite gut [50–52]. To date, several bacteria have been isolated and identified from the termite gut systems, including *Acetonema longum* and *Clostridium mayombei* from *Macrotermes gilvus* [53]. Recently, we have also isolated several bacteria from the gut regions of fungus-growing termites and wood-feeding *Coptotermes formosanus*, capable of degrading cellulose, hemicellulose, and lignin analogue dyes [4,6].

Spirochaetes are by far the most prevalent and species-rich bacteria in wood-feeding termites. Owing to their high surface-to-volume ratio and free-swimming nature, spirochaetes found in the hindgut of termites’ can circumvent the restrictions of metabolic diffusion in microoxic/anoxic habitats [54]. While most of the bacteria in lower termites reside in the cytoplasm or attach externally to the flagellate cells [49], certain tiny bacteria adhere to the cuticle or filamentous microorganisms, which are in turn affixed with the wall of the hindgut [55].

However, the diversity of bacterial species varies with respect to the termite species; for example, *Coptotermes* species are dominated by the members of Bacteroidetes [50], whereas Candidatus (Elusimicrobia: Endomicrobia) are predominant in *Reticulitermes* spp. [27,51,56]. Like *Coptotermes* termites, *Odontotermes* and *Macrotermes* species are also dominated by the Bacteroidetes and Firmicutes [57].

The Nasutitermitinae and Termitinae workers contain a significant amount of fibrobacters belonging to the phylum TG3 [58]. These evolutionary changes in the bacterial diversity allowed for the termites to adapt to diverse habitats and diets while restricting the lower termites to wood-feeding habits [27]. Termites harbor a diverse array of microbiota, the majority of which are unculturable, with many taxa still unknown. Consequently, the mystery of termite–microbe symbiosis is still at its nascent stage. However, a genome-based analysis of the unculturable bacteria appears as a strong approach to address this issue.

Table 1. Diversity of the bacteria isolated and identified from the gut systems of termites.

Bacterial Genera	Prokaryote Type	Termite Species	Gut-Region	References
<i>Streptomyces naraensis</i>	Actinomycete	<i>Coptotermes formosanus</i>	Whole gut	[59]
<i>S. filamentosus</i>		<i>Odontotermes formosanus</i>		[60]
<i>Clostridium mayombeii</i> , <i>Sporomusa termitida</i> , <i>Klebsiella variicola</i> , <i>Acetonema longum</i> <i>K. pneumoniae</i> , <i>M. cuticularis</i> , <i>M. curvans</i> , <i>M. filiformis</i>	Bacteria	<i>Nasutitermes nigriceps</i>	Whole gut	[61]
		<i>Pterotermes occidentis</i>		
	Archaea	<i>O. formosanus</i>	Whole gut	[62]
<i>Treponema isoptericolens</i> , <i>Spirochaeta coccoides</i>	Spirochete	<i>Reticulitermes speratus</i>	Whole gut	[62]
		<i>Cubitermes ugandensis</i>	Whole gut	[63]
<i>Acinetobacter seifertii</i> , <i>Enterobacter asburiae</i> , <i>E. cloacae</i> , <i>Lysinibacillus macrolides</i> ,	Bacteria	<i>C. formosanus</i>	Foregut	[4]
<i>S. marcescens</i> , <i>P. stutzeri</i> , <i>S. hominis</i> , <i>B. cereus</i> , <i>K. aerogenes</i> , <i>E. hormaechei</i>	Bacteria	<i>C. formosanus</i>	Foregut Midgut Hindgut	[6]
<i>E. cancerogenes</i> , <i>E. ludwigii</i> , <i>L. boronitolerans</i> , <i>Lysinibacillus</i> sp., <i>P. fluorescens</i> , <i>P. plecoglossicida</i> , <i>P. putida</i>	Bacteria	<i>C. formosanus</i>	Midgut	[3]
<i>A. calcoaceticus</i> , <i>B. simplex</i> , <i>Dietza</i> sp., <i>E. mori</i> , <i>L. fusiformis</i> , <i>P. nitroreducens</i>	Bacteria	<i>C. formosanus</i>	Hindgut	[3]
<i>Bacillus</i> spp.	Bacteria			
<i>Paenibacillus lactis</i> AFC1				
<i>L. fusiformis</i> AFC2				
<i>Stenotrophomonas maltophilia</i> AFC3	Bacteria	<i>Psammotermes hypostoma</i>	Whole gut	[65]
<i>L. macrolides</i> AFC4				
<i>Bacillus cereus</i> AFC5				
<i>Bacillus</i> spp., <i>Paenibacillus</i> spp.	Bacteria	<i>R. lucifugus</i>	Whole gut	[66]
<i>Cellulomonas/Oerskovia</i> , <i>Microbacterium</i> and <i>Kocuria</i>	Actinomycete	<i>Z. angusticollis</i>		
<i>Bacillus</i> , <i>Brevibacillus</i> , <i>Paenibacillus</i> , <i>fipia</i> , <i>Agrobacterium/Rhizobium</i> , <i>Brucella/Ochrobactrum</i> , <i>Pseudomonas</i> and <i>Sphingomonas/Zymomonas</i>	Bacteria	<i>Z. angusticollis</i>	Whole gut	[62]

Table 1. Cont.

Bacterial Genera	Prokaryote Type	Termite Species	Gut-Region	References
<i>Citrobacter farmeri</i>		<i>C. formosanus</i>	Whole gut	[67]
		<i>O. formosanus</i>		
<i>Bacillus</i> spp.	Bacteria	<i>Mastotermes darwiniensis</i> , <i>Cryptotermes primus</i> , <i>N. arborum</i> , <i>Thoracotermes macrothorax</i> , <i>Anoplotermes pacificus</i>	Whole gut	[68]
<i>Isoptericola variabilis</i>		<i>Mastotermes darwiniensis</i>	hindgut	[69,70]
<i>Sporomusa aerivorans</i>		<i>Thoracotermes macrothorax</i>	Whole gut	[71]
<i>Candidatus Vestibaculum illigatum</i>		<i>Neotermes cubanus</i>	Whole gut	[51]
<i>T. azotonutricium</i>	Spirochaetes	<i>Zootermopsis angusticollis</i>	Whole gut	[72]
<i>T. primitia</i>				
<i>Candidatus Endomicrobium trichonymphae</i>		<i>R. santonensis</i>	Whole gut	[51]
<i>Candidatus Endomicrobium pyrsonymphae</i>				

7.2. Symbiotic Flagellates

The presence of flagellates is typically represented in the gut system of lower termites only, suggesting their evolutionary adaptations. In particular, the enlarged hindgut shelters a high number of protists that share symbiotic connection, offering two-way benefits to the host (Table 2). They are primarily responsible for the enzyme secretions to hydrolyze the cellulose and hemicellulose molecules. Second, they provide a large surface area for the colonization of ecto- as well as endo-symbiotic bacteria within the termite gut systems [73]. Due to their important support in the lignocellulose breakdown, protists are essential to the survival of the termite host. Cleveland in 1924 was the first to show that *Reticulitermes flavipes* could not consume cellulose and perished within 20 days after defaunation of intestinal protists [33]. To metabolize their cellulose-rich diet, protists secrete and express their own cellulases. This allows them to digest and break down the cellulose components and convert them into nutrients and energy [74].

The symbiotic protists in termites express several genes that encode cellulose- and hemicellulose-degrading enzymes belonging to different Glycoside Hydrolase Families (GHF). Several meta-transcriptomic investigations confirmed the involvement of these genes in lignocellulose bioconversion and metabolism [75,76]. Almost 1 in every 10 expressed genes of protists in termites are responsible for cellulose degradation. Moreover, the occurrence of the GH7 family cellulases in all the gut protists suggested them as “core enzyme set” in termites. Despite the uneven expression levels of glycosyl hydrolases, the GHF7 shows highest expression in termite guts. Among the expression of 1000 clones of an environmental expressed sequence tags (EST) reported in the *R. flavipes* protist community, 6.2% of the sequences corresponded to GHF7. This family contains cellobiohydrolase (CBH) and endoglucanase (EG) subtypes of cellulases. The GHF7 CBHs make up to 4.1% of all ESTs, whereas EGs occupy 2.1% only. The elevated expression of these enzymes in wood-feeding termites implies that they are crucial for the metabolism of cellulose. Additionally, GHF45-related protist cellulases have been discovered in *Reticulitermes speratus* and *Mastotermes darwiniensis* [77,78]. Many other hemicellulose degrading enzymes, such as xylanases and mannanases, are also reported from symbiotic protists. They comprised mannanases from GHF47, GHF26, β -galactosidases (GHF42), xylanases (GHF8, GHF10, GHF11, GHF43, and GHF62), and xylosidases from GHF5 [75,79]. Since hemicellulose covers and protects the cellulose content of the plant matter, the degradation of hemicellu-

lose is imperative for exposing the polymer chains for enzymatic attacks. Over the course of evolution, symbiotic protists have developed complex glycosyl hydrolases to extract carbon and energy from recalcitrant lignocellulose. Although several cellulases and hemicellulases have been reported from the protist community, little information is available about the role of these symbionts in lignin degradation. This lacuna might be compensated by the initial mechanical grinding and enzymatic pretreatment by host termites to complete the metabolism of lignocellulose [80]. Further the protists of the lower termites are also known to hydrolyze chitin and prevent infection from environmental pathogens, including entomopathogenic fungi [81].

Table 2. List of the protists reported in the gut systems of termites.

Termite Host	Protist/Flagellate				References
	Class	Order	Family	Genus	
<i>Incisitermes minor</i>	Trichomonadea	Trichomonadida	Devescovinidae	<i>Metadevescovina cuspidata</i>	[75,79]
<i>Coptotermes</i> spp.	Parabasalia	Spirotrichonymphida	Holomastigotoididae	<i>Holomastigotoides</i>	[82–84]
		Trichonymphida	Teranymphidae	<i>Pseudotriconympha</i>	
			Trichonymphidae	<i>Spirotrichonympha</i> <i>Cononympha</i>	
<i>Reticulitermes</i> spp.	Parabasalia	Spirotrichonymphida	Holomastigotoididae	<i>Holomastigotoides</i>	[85,86]
		Trichonymphida	Trichomonadidae	<i>Trichomonas</i>	
		Teranymphidae	<i>Teranympha</i>		
		Trichonymphidae	<i>Spirotrichonympha</i> <i>Trichonympha</i>		
	Preaxostyla	Tritrichomonadida	Monocercomonadidae	<i>Monocercomonas</i>	
		Oxymonadida	Pyrsonymphidae	<i>Dinenympha</i> <i>Pyrsonympha</i>	
<i>Rhinotermes</i> spp.	Parabasalia	Cristamonadida	Lophomonadidae	<i>Gigantomonas</i>	[82,87]
<i>Schedorhinotermes</i> spp.	Parabasalia	Trichonymphida	Teranymphidae	<i>Pseudotriconympha</i>	[88]
<i>Cryptotermes</i> spp.	Parabasalia	Cristamonadida	Lophomonadidae	<i>Stephanonympha</i> <i>Devescovina</i>	[86,89]
<i>Epiclotermes</i> spp.	Parabasalia	Trichonymphida	Staurojoeninidae	<i>Staurojoenina</i>	[90]
<i>Glyptotermes</i> spp.	Parabasalia	Cristamonadida	Lophomonadidae	<i>Devescovina</i> <i>Macrotrichomonas</i>	[86]
<i>Incisitermes</i> spp.	Parabasalia	Cristamonadida	Lophomonadidae	<i>Coronympha</i>	[84,86]
		Trichonymphida	Staurojoeninidae	<i>Staurojoenina</i>	
			Trichonymphidae	<i>Trichonympha</i>	
<i>Kalotermes</i> spp.	Parabasalia	Cristamonadida	Lophomonadidae	<i>Calonympha</i> <i>Devescovina</i> <i>Joenia</i>	[84]
		Tritrichomonadida	Monocercomonadidae	<i>Stephanonympha</i> <i>Monocercomonas</i>	
<i>Neotermes</i> spp.	Parabasalia	Cristamonadida	Lophomonadidae	<i>Devescovina</i> <i>Foaina</i>	[91]
	Preaxostyla	Oxymonadida	Oxymonadidae	<i>Oxymonas</i>	
<i>Archotermopsis</i> spp.	Parabasalia	Honigbergiellida Trichonymphida	Honigbergiellidae Teranymphidae	<i>Ditrichomonas</i> <i>Pseudotriconympha</i>	[92]
<i>Hodotermopsis</i> spp.	Parabasalia	Spirotrichonymphida	Holomastigotoididae	<i>Spirotrichonymphella</i>	[85,93]
		Trichomonadida	Trichomonadidae	<i>Trichomonas</i>	
		Hoplonymphidae	<i>Hoplonympha</i>		
		Teranymphidae	<i>Eucomonympha</i>		
		Trichonymphidae	<i>Spirotrichonympha</i> <i>Trichonympha</i>		
<i>Porotermes</i> spp.	Parabasalia	Cristamonadida	Lophomonadidae	<i>Joenina</i>	[94,95]
		Spirotrichonymphida	Holomastigotoididae	<i>Spirotrichonymphella</i>	
		Trichomonadida	Trichomonadidae	<i>Pseudotrypanosoma</i>	
		Trichonymphida	Teranymphidae Trichonymphidae	<i>Pseudotriconympha</i> <i>Trichonympha</i>	

Table 2. Cont.

Termite Host	Protist/Flagellate				References
	Class	Order	Family	Genus	
<i>Zootermopsis</i> spp.	Parabasalia	Hypotrichomonadida	Hypotrichomonadidae	<i>Trichomitus</i>	[89,96]
	Preaxostyla	Trichonymphida Oxymonadida	Trichonymphidae Streblomastigidae	<i>Trichonympha</i> <i>Streblomastix</i>	
<i>Hodotermes</i> spp.	Parabasalia	Cristamonadida	Lophomonadidae	<i>Devescovina</i> <i>Foaina</i>	[97,98]
		Trichonymphida	Spirotrichosomidae	<i>Gigantomonas</i> <i>Stephanonympha</i> <i>Leptospirotrichomyxa</i>	
<i>Mastotermes</i> spp.	Parabasalia	Cristamonadida	Lophomonadidae	<i>Deltotrichonympha</i> <i>Koruga</i>	[99–101]
		Trichomonadida	Trichomonadidae	<i>Metadevescovina</i> <i>Mixotricha</i> <i>Pentatrichomonoides</i>	

7.3. Symbiotic Fungi

Termites share an intricate relation with fungi. Hitherto, a number of fungal and yeast species have been observed in the gut systems of termites. Fungus-growing termites are prevalent in the tropical regions of Asia and Africa [41]. Higher termites from the subfamily Macrotermitinae coexist with the fungus, *Termitomyces* spp. It is well acknowledged that the fungal symbionts play a significant role in the breakdown of lignocellulose, thus aiding the host termites. In fungus-growing termites, young workers ingest *Termitomyces* nodules along with LCB and excrete lignin-rich feces to build fresh fungus combs [102]. Within 40 days, *Termitomyces* converts the fresh comb into a well-decomposed mature comb (old comb), which is subsequently ingested by old worker termites. The enzymatic contribution from the termite host, endosymbiont (gut microbiota), and exosymbiont (*Termitomyces*) greatly facilitates the degradation of plant biomass in fungus-growing termites [6]. Further, the symbiosis of termites and fungi for lignocellulose bioconversion is confirmed in two ways. First, the biochemical detection reveals an apparent increase in the C-to-N ratio and higher nitrogen quality in certain fungus combs. Second, the identification and expression of laccase genes in the genome of symbiotic *Termitomyces* spp., found in fungus-growing termites, also demonstrates lignin the degradation capacity of the fungal symbionts [103]. The breakdown of the lignin barrier apparently allows access for glycosyl hydrolases to attack cellulose and hemicellulose, thereby increasing the overall degradation. Third, according to a subtractive EST study of the cultured *Termitomyces* spp. of *Macrotermes gilvus* [104], *Termitomyces* releases a variety of cellulolytic or hemicellulolytic enzymes to break down plant polysaccharides in the termite nests. The cDNA library of the termite revealed a high expression of genes encoding cellulose (EG and CBH), hemicellulose (endo-1, 4-b-xylanases, β -mannanase, etc.), and pectin (endo-polygalacturonase, exo-polygalacturonase) as well as pectate lyase (PL) and rhamnogalacturonan lyase, implicated in lignocellulose degradation. These fungal enzymes belong to the CAZy families, such as GH6, GH7, and GH61 (cellulases); GH11 (xylanase); and the pectinases of PL2 and PL4. The partially digested plant material generated from the fungal combs of worker termites is exposed to gut microbiota for further digestion. The members of the genera, such as *Ascomycota*, *Bysochlamys*, *Spiromastox*, and *Malassezia*, have been defined as core microbiota in *Microcerotermes strunckii*, *Nasutitermes corniger*, and *Termes riograndensis* [105].

8. Biorefinery Potential of Termite Symbiosis

Recently, termites have attracted a lot of attention from scientists and academicians due to their pest nature as well as symbiosis for lignocellulose digestion. Correspondingly, the termite research has produced a wealth of information with several biotechnological applications. The highly explored knowledge of lignocellulose digestion by termites along with their metabolic and physicochemical processes has provided a basis for the

redesign of novel and efficient bioreactors for the fermentation of LCB into bioenergy. The wood-feeding termites apply a unique and stepwise process for the hydrolysis of lignin, hemicellulose, and cellulose via biocatalytic processes (Figure 5); therefore, mimicking their digestive metabolism and physiochemical gut environment will lay the foundation for a nature-inspired lignocellulose processing system. The underexplored biodiversity and biochemistry of the termite guts represent a promising resource of novel catalytic processes [26]. Investigating diverse termite lignocellulolytic systems will undoubtedly unveil numerous genes encoding novel biocatalysts along with their expression systems and associated mechanisms, providing valuable insights for the innovative design of nature-inspired technology. Despite a century-old research area, termite biology has recently evolved and developed into a multidisciplinary area that will pave the way for innovations and future breakthroughs in the bioconversion of lignocellulose for biofuels. Termite biotechnology involves biomimetics of the termite gut for future biorefinery apart from other industrial applications.

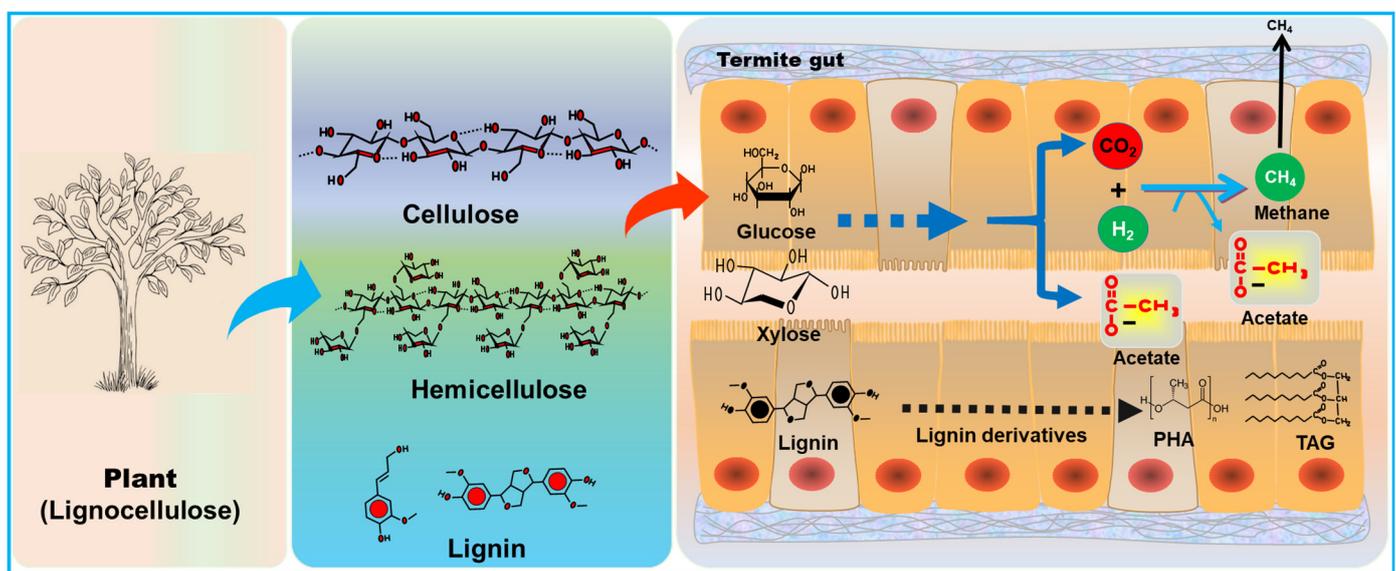


Figure 5. An overview of the metabolic pathways employed by the termite holobiome community for the digestion of lignocellulose within the gut systems. PHA: Polyhydroxyalkanoate; TAG: Triacyl glycerate.

In recent years, significant and encouraging progress has been achieved in the field of “biomimetics”, particularly the design of novel reactors simulated from the pretreatment as well as the hydrolysis and fermentation of LCB by wood-feeding termites [106]. Droge et al. cultured a spirochete isolated from the hindgut of a lower dry-wood termite, *Neotermes castaneus*, on different carbon sources [64]. After evaluation, the spirochetes were found to produce acetate, formate, and ethanol from lignocelluloses. In lower termites, similar bacteria can degrade cellulose into acetic acid and are known to function for hydrogen consumption [72,107]. These spirochetes have been isolated from five species of termites, viz, *Cryptotermes cavifrons*, *Kaloterme flavicollis*, *Heterotermes tenuis*, *Neotermes mona*, and *Reticulitermes grassei*, that feed on dry-wood, indicating their coevolution with host termites [108]. Acetic acid production by the symbiotic bacteria is also reported in termites [57]. The gut symbionts, such as *Sporomusa termitida*, *Acetonema longum*, and *Clostridium mayombi*, are known to contribute 1/3 of the acetic acid for termite respiration [109]. Since *Lactococci* and *Enterococci* are well-known as cellobiose and xylose fermenters, they may be responsible for acetic acid production [110]. Hydrogen is also released by higher termites, where protists and bacterial fermentations are noted to produce molecular hydrogen (H₂) [111]. For example, numerous 16S rRNA genes isolated from the gut system of *C. orthognathus* [112] exhibit close relation to genes from the cellulolytic, hydrogen-producing bacterium, *Clostridium termitidis* [26]. Similarly, Kane and Breznak [113] discovered an acetogenic *Clostridium mayombe* from soil-feeding *Cubitermes*

speciosus. The obligate anaerobe was found to produce approximately 13.4 mmol/100 mmol of glucose, stating 85% carbon recovery by the bacterium.

The termite gut exhibits a daily turnover rate of hydrogen, ranging from 9 to 33 m³ per m³ of termite hindgut [109]. Notably, when compared to rumens, the termite paunch is significantly smaller, being 10⁸ times smaller. This size difference leads to a substantial increase in oxygen influx (500 times) per unit volume. The transit time for ingested forage in termite guts is remarkably short, only taking one day. This efficient process makes bio-mimicking termite gut microbes through in vitro co-culturing relatively straightforward. Mathew et al. [114] studied the synergistic relationship of termite gut symbionts for hydrogen production. The researcher's biomimicked the termite gut environment by co-culturing isolates from *O. formosanus*, specifically *Bacillus* and *Clostridium* sp., in batch modes using different carbon sources. The study reveals that the mutualistic interaction of *Bacillus* sp. created an anaerobic condition conducive to the growth of *Clostridium*, resulting in the maximum production of hydrogen (4.08 mmol/mL of hydrogen using glucose as a substrate). Theoretically, the gut bacterial symbionts of termites can convert a sheet of A4 paper into two liters of hydrogen, which can serve as an ideal inorganic energy resource [26]. Some estimations have stipulated that the subterranean termites can produce 3858 ± 294 μmol of molecular hydrogen per gram of cellulose, suggesting the unique and high efficiency of the termite gut for the generation of H₂ from cellulosic substrates [115].

Earlier studies have reported some bacterial strains, such as *Clostridium* sp., *Enterobacter cloacae*, and *E. aerogenes*, from the termite gut can serve as facultative and obligate anaerobes, producing biohydrogen through dark fermentation (Table 3) [116]. These strict anaerobes are considered highly effective candidates for H₂ productions. These enteric bacteria, for example, *E. cloacae* KBH3, even function under the microoxic conditions of the gut systems, utilizing the available oxygen for H₂ production [117]. The *E. cloacae* KBH3 exhibited a high production rate of approximately 180.74 mL H₂/L/h, and the hydrogen yield was 1.8 mol H₂/mol of glucose under batch fermentation. The cumulative hydrogen production increased, attributed to the subsequent consumption of formate by *E. cloacae* KBH3 [118]. The bacteria, such as *Sporoniusa termitida* and *Acetonema longum*, isolated from the wood-feeding termites, *Nasutitermes nigriceps* and *Pterotermes occidentis*, are also used to understand the competitive mechanisms of gut acetogens for in situ hydrogen production. Ramachandran et al. [119] investigated the fermentative pattern of *Clostridium termitidis* CT1112, isolated from *Nasutitermes lujae*, for hydrogen production under batch cultivation. The study utilized α-cellulose and cellobiose as sole carbon sources, and the fermentative end products included ethanol, CO₂, acetate, lactate, and formate. As an obligate anaerobe, *Clostridium termitidis* exhibited a maximum yield of approximately 4.6 mmol/L and 7.7 mmol/L of H₂ for cellobiose and α-cellulose, respectively. This highlights the dependence of termite nutrition on bacterial acetogenesis for acetate oxidation to meet respiratory requirements [120]. Despite these reports, the roles of the bacteria represented by these and various other unculturable lineages from termites in cellulose degradation and hydrogen production awaits further exploration. Ongoing global studies are focused on optimizing fermentation for H₂ production from gut symbionts to enhance net energy yield and production rates.

Table 3. List of the symbiotic microbes isolated from the termite gut systems used for the biofuel or biohydrogen productions.

Microorganism	Termite Host	Biofuel Type	Carbon Source	References
<i>Bacteroides</i> , <i>Prevotella</i>	<i>N. ephratae</i>	Biomethane	Wheat straw	[121]
<i>Streptomyces</i> sp.	<i>Microcerotermes</i> species	Bioethanol	Wheat straw	[122]
<i>Bacillus</i> sp. BMP01, <i>Ochrobactrum oryzae</i> BMP03	<i>Cryptotermes brevis</i>	Bioethanol	CMC, Xylan, Lignin	[123]
Bacterial symbionts	<i>Nasutitermes ephratae</i> , <i>Microcerotermes parvus</i> , <i>N. lujae</i> , <i>Termes hospes</i>	Biomethane	Wheat straw	[124]

Table 3. Cont.

Microorganism	Termite Host	Biofuel Type	Carbon Source	References
Actinobacteria	<i>M. nervosus</i> , <i>Macrognathothermes sunteri</i> , <i>Tumulitermes pastinatio</i>	Hydrogen, Biomethane	Organic Carbon	[125]
Symbionts	<i>Reticulitermes speratus</i>	Hydrogen	Wood	[126]
<i>Treponema primitia</i>	<i>Zootermopsis angusticollis</i>	Acetate	Wood	[127]
<i>Clostridium termitidis</i> , <i>Clostridium beijerinckii</i>	<i>Nasutitermes</i> spp.	Hydrogen	Cellulose	[128]
<i>Methylocella</i> sp., and other symbionts	<i>R. speratus</i> , <i>Z. nevadensis</i> , <i>Cubitermes orthognathus</i>	Biomethane, Hydrogen	Wood	[120,129,130]
<i>Enterococcus</i> sp.	<i>R. flavipes</i>	Acetate	Lignocellulose	[131]
<i>Sporomusa termitida</i> , <i>Sporomusa</i> sp. strain TmA03	termites	Hydrogen	Lignocellulose	[71]
Microbiota	<i>Cubitermes</i> spp.	Biomethane	Lignocellulose	[132]
<i>Sporomusa aerivorans</i>	<i>Thoracotermes macrothorax</i>	Hydrogen	Lactate	[133]
<i>Sporotalea propionica</i>	<i>T. macrothorax</i>	Hydrogen	Glucose	[134]
<i>Acetonema longum</i>	<i>Pterotermes occidentis</i>	Hydrogen	Yeast extract, Rumen fluid, Resazurin	[113]
<i>Enterobacter cloacae</i> KBH3	<i>Globitermes</i> sp.	Hydrogen	Glucose	[118]
<i>Pseudotriconympha grassii</i>	<i>C. formosanus</i>	Hydrogen	Wood cellulose	[111]
<i>Trichonympha sphaerica</i>	<i>Z. termites</i>	Hydrogen	Lignocellulose	[135]
<i>Trichomitopsis termopsidis</i> , <i>Hexamastix termopsidis</i> and <i>Tricercomitus termopsidis</i> , Gut microbiota	<i>R. santonensis</i> , <i>Z. nevadensis</i> , <i>Cryptotermes secundus</i>	Hydrogen, Biomethane	CO ₂ , Formate, Lactate	[130]
<i>Trichomitopsis termopsidis</i> , <i>Trichonympha sphaerica</i>	<i>Z. termites</i>	Hydrogen, Biomethane	Cellulose, Corncob, Cereal leaves	[136]

CMC: CarboxyMethyl Cellulose.

9. Challenges and Future Perspectives

Due to the depletion of fossil fuels, biofuels are now seen as renewable energy resources. The gut microbial symbionts of termites with their lignocellulolytic proficiencies could be a “treasure box” with enormous biotechnological prospects for meeting the world’s energy needs through greater bioenergy production. Although the advent of modern technologies for modifying enzyme properties is rapidly gaining favor to harness novel cellulases and hemicellulases, the bioprospection and screening of wild strains needs to be continued, as nature offers the most sophisticated tool of natural selection, i.e., evolution. Similar endeavors are expected to target both diversity-rich environments and extremophile niches, such as termite gut systems, for the identification of novel microbial producers. To discover the microbial populations involved in the production of biogas and bioethanol, advanced “omics” methods, such as metagenomics, metatranscriptomics, meta-proteomics, and metabolomics, need to be frequently applied.

Although the area of termite–bacterial research that describes the 16S rRNA gene profiles of gut communities is flourishing, with the majority of the studies providing a single picture of the whole gut systems, the studies do not estimate the numbers of bacterial cells present in respective gut regions particularly in the foregut and midgut. A complete picture for a better understanding of the actual densities and stability of the gut microbiota is paramount for a comprehensive analysis of the gut region-specific diversity data. Furthermore, laboratory surveys and experimental studies may not provide an accurate picture of the gut community functions or roles, as they exist under natural populations. For instance, the functions of gut microorganisms can be elucidated only if the proper environmental conditions mimicking the gut habitats is maintained. Keeping in mind the pace of the current research and advances in technologies, in next few years, we will likely discover a lot more about how termite symbiosis functions towards the

maximum utilization and metabolism of LCB. Such research will help to design novel bioreactors for the efficient bioconversion of LCB into bioenergy.

The highly advanced and high throughput sequencers have provided huge strides to gain insights into genomes of novel and unculturable bacteria through metagenomics. Correspondingly a diverse array of cellulase genes is now available from the ever-expanding genomic libraries. Further, the deconstruction of recalcitrant lignocellulose will require integrated efforts based on the four strategies: (1) deciphering the novel cellulase genes through culturable and culture-independent strategies; (2) the advanced design of bioreactors by mimicking the natural biomass utilization systems; (3) the structural and functional analysis involving molecular mechanism of cellulases and their interaction with substrates that can be exploited for designing novel cellulases through protein and metabolic engineering; and (4) the integration of biological, mathematical, and biophysical disciplines to achieve the accelerated production of lignocellulases. These challenges can be accomplished through a full integration of the academic, government, and industry efforts in teams that can create and apply new knowledge to key cost drivers.

The key take-home messages that can be adapted from this review regarding termite–microbial symbiosis for the advanced design of future biorefinery can include the following:

Efficient Biomass Conversion: Termites have evolved efficient enzymatic and microbial systems in their guts to break down lignocellulosic biomass, including cellulose, hemicellulose, and lignin. This can provide insights into designing enzymatic and microbial processes for effective biomass conversion in biorefineries.

- I. **Synergistic Microbial Consortia:** Termites rely on a symbiotic relationship with their gut microbiota to efficiently digest complex plant materials. Understanding the composition and interactions of microbial communities within gut systems can inspire the development of microbial consortia for optimized biomass degradation and conversion in biorefineries.
- II. **Enzyme Discovery and Engineering:** The gut microbiota of termites produces several enzymes, including cellulases, ligninases, and hemicellulases, essential for biomass degradation. These enzymes can be studied and potentially engineered to improve enzyme efficiency and specificity for biorefinery applications.
- III. **Biofuel Production:** Termites can produce methane and acetate as byproducts of biomass digestion through enteric fermentations. This can provide insights into enhancing methane production or other biofuel production pathways in biorefineries.
- IV. **Nutrient Recovery and Recycling:** Termites efficiently extract nutrients from plant material in their digestive process. This knowledge can be used to design processes for nutrient recovery and recycling in biorefineries, minimizing waste and maximizing resource utilization.
- V. **Ecosystem Integration and Circular Economy Principles:** Studying termites' role in their ecosystem and how they contribute to nutrient cycling and soil enrichment can provide inspiration for incorporating circular economy principles in biorefineries to minimize waste and promote sustainability.

10. Conclusions

To conclude, termites can indeed serve as models for future biorefineries due to their remarkable ability to efficiently break down and utilize lignocellulosic biomass for energy and nutrient cycling. Termites have a specialized gut microbiota, including bacteria, archaea, and protozoa, that play a crucial role in breaking down complex plant materials into simpler compounds. By studying and learning from termites and their gut microbiota, researchers and engineers can develop innovative strategies and technologies for sustainable and efficient biorefineries, ultimately contributing to a more sustainable and bio-based economy.

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