

Termite Microbial Symbiosis for Lignocellulosic Future Biorefinery

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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.

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

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.

2. 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 ^[10]. Cellulose being a macromolecule is cleaved by the bacterial enzymes into short-chain fatty acids in the gut system of termites ^[11]. 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 ^{[12][13][14]}.

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 [13][15]. 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.

2.1. 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 [16]. 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 [17]. 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][18][19]. Bacteria belonging to four major phyla, such as Elusimicrobia, Bacteroidetes, Proteobacteria, and Actinobacteria, are found as endosymbionts in protist cells within the termite gut [20][21][22]. To date, several bacteria have been isolated and identified from the termite gut systems, including *Acetonema longum* and *Clostridium mayombeii* from *Macrotermes gilvus* [23].

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 [24]. While most of the bacteria in lower termites reside in the cytoplasm or attach externally to the flagellate cells [17], certain tiny bacteria adhere to the cuticle or filamentous microorganisms, which are in turn affixed with the wall of the hindgut [25]. However, the diversity of bacterial species varies with respect to the termite species; for example, *Coptotermes* species are dominated by the members of Bacteroidetes [20], whereas *Candidatus* (Elusimicrobia: Endomicrobia) are predominant in *Reticulitermes* spp. [18][21][26]. Like *Coptotermes* termites, *Odontotermes* and *Macrotermes* species are also dominated by the Bacteroidetes and Firmicutes [27].

The Nasutitermitinae and Termitinae workers contain a significant amount of fibrobacteres belonging to the phylum TG3 [28]. 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 [18]. 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	[29]
<i>S. filamentosus</i>		<i>Odontotermes formosanus</i>		[30]
		<i>Nasutitermes nigricipes</i>		
<i>Clostridium mayombeii</i> , <i>Sporomusa termitida</i> , <i>Klebsiella variicola</i> , <i>Acetonema longum</i>	Bacteria	<i>Pterotermes occidentis</i>	Whole gut	[31]
<i>K. pneumoniae</i> , <i>M. cuticularis</i> , <i>M. curvans</i> , <i>M. filiformis</i>		<i>O. formosanus</i>		
	Archaea	<i>Reticulitermes speratus</i>	Whole gut	[32]
		<i>Cubitermes ugandensis</i>	Whole gut	[33]
<i>Treponema isoptericolens</i> , <i>Spirochaeta coccoides</i>	Spirochete	<i>Incisitermes tabogae</i>	Whole gut	[34]

Bacterial Genera	Prokaryote Type	Termite Species	Gut-Region	References
<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>Dietzia</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	[35]
<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	[36]
<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	[32]
<i>Citrobacter farmeri</i>		<i>C. formosanus</i>	Whole gut	[37]
		<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	[38]
<i>Isoptericola variabilis</i>		<i>Mastotermes darwiniensis</i>	hindgut	[39][40]
<i>Sporomusa aerivorans</i>		<i>Thoracotermes macrothorax</i>	Whole gut	[41]
<i>Candidatus Vestibaculum illigatum</i>		<i>Neotermes cubanus</i>	Whole gut	[21]
<i>T. azotonutricium</i>	<i>Spirochaetes</i>	<i>Zootermopsis angusticollis</i>	Whole gut	[42]
<i>T. primitia</i>				
<i>Candidatus Endomicrobium trichonymphae</i> ,		<i>R. santonensis</i>	Whole gut	[21]

2.2. 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 [43]. 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 [44]. 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 [45].

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 [46][47]. 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* [48][49]. 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 [46][50]. Since hemicellulose covers and protects the cellulose content of the plant matter, the degradation of hemicellulose 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 [51]. Further the protists of the lower termites are also known to hydrolyze chitin and prevent infection from environmental pathogens, including entomopathogenic fungi [52].

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	Devescovichidae	<i>Metadevescovina cuspidata</i>	[46][50]
		Spirotrichonymphida	Holomastigotoididae	<i>Holomastigotoides</i>	
			Teranymphidae	<i>Pseudotrichonympha</i>	
<i>Coptotermes</i> spp.	Parabasalia	Trichonymphida	Trichonymphidae	<i>Spirotrichonympha</i>	[53][54][55]
				<i>Cononympha</i>	
		Spirotrichonymphida	Holomastigotoididae	<i>Holomastigotoides</i>	
			Trichomonadidae	<i>Trichomonas</i>	
			Teranymphidae	<i>Teranympha</i>	
<i>Reticulitermes</i> spp.	Parabasalia	Trichonymphida	Trichonymphidae	<i>Spirotrichonympha</i>	[56][57]
				<i>Trichonympha</i>	
		Tritrichomonadida	Monocercomonadidae	<i>Monocercomonas</i>	
				<i>Dinenympha</i>	
	Preaxostyla	Oxymonadida	Pyrsonymphidae	<i>Pyrsonympha</i>	
<i>Rhinotermes</i> spp.	Parabasalia	Cristamonadida	Lophomonadidae	<i>Gigantomonas</i>	[53][58]
<i>Schedorhinotermes</i> spp.	Parabasalia	Trichonymphida	Teranymphidae	<i>Pseudotrichonympha</i>	[59]
<i>Cryptotermes</i> spp.	Parabasalia	Cristamonadida	Lophomonadidae	<i>Stephanonympha</i>	[57][60]
				<i>Devescovina</i>	
<i>Epicalotermes</i> spp.	Parabasalia	Trichonymphida	Staurojoenidae	<i>Staurojoenina</i>	[61]

Termite Host	Protist/Flagellate				References
	Class	Order	Family	Genus	
<i>Glyptotermes</i> spp.	Parabasalia	Cristamonadida	Lophomonadidae	<i>Devescovina</i>	[57]
				<i>Macrotrichomonas</i>	
<i>Incisitermes</i> spp.	Parabasalia	Cristamonadida	Lophomonadidae	<i>Coronympha</i>	[55][57]
		Trichonymphida	Staurojoeninidae	<i>Staurojoenina</i>	
			Trichonymphidae	<i>Trichonympha</i>	
				<i>Calonympha</i>	
<i>Kalotermes</i> spp.	Parabasalia	Cristamonadida	Lophomonadidae	<i>Devescovina</i>	[55]
				<i>Joenia</i>	
				<i>Stephanonympha</i>	
		Tritrichomonadida	Monocercomonadidae	<i>Monocercomonas</i>	
<i>Neotermes</i> spp.	Parabasalia	Cristamonadida	Lophomonadidae	<i>Devescovina</i>	[62]
				<i>Foaina</i>	
<i>Archotermopsis</i> spp.	Parabasalia	Oxymonadida	Oxymonadidae	<i>Oxymonas</i>	[63]
		Honigbergiellida	Honigbergiellidae	<i>Ditrichomonas</i>	
		Trichonymphida	Teranymphidae	<i>Pseudotrichonympha</i>	
		Spirotrichonymphida	Holomastigotoididae	<i>Spirotrichonymphella</i>	
<i>Hodotermopsis</i> spp.	Parabasalia	Trichomonadida	Trichomonadidae	<i>Trichomonas</i>	[56][64]
			Hoplonymphidae	<i>Hoplonympha</i>	
			Teranymphidae	<i>Eucomonympha</i>	
		Trichonymphida		<i>Spirotrichonympha</i>	
			Trichonymphidae	<i>Trichonympha</i>	
				<i>Trichonympha</i>	
<i>Porotermes</i> spp.	Parabasalia	Cristamonadida	Lophomonadidae	<i>Joenina</i>	[65][66]
		Spirotrichonymphida	Holomastigotoididae	<i>Spirotrichonymphella</i>	
		Trichomonadida	Trichomonadidae	<i>Pseudotrypanosoma</i>	
		Trichonymphida	Teranymphidae	<i>Pseudotrichonympha</i>	
<i>Zootermopsis</i> spp.	Parabasalia		Trichonymphidae	<i>Trichonympha</i>	[60][67]
		Hypotrichomonadida	Hypotrichomonadidae	<i>Trichomitus</i>	
		Trichonymphida	Trichonymphidae	<i>Trichonympha</i>	
<i>Hodotermes</i> spp.	Parabasalia	Oxymonadida	Streblomastigidae	<i>Streblomastix</i>	[68][69]
				<i>Devescovina</i>	
		Cristamonadida	Lophomonadidae	<i>Foaina</i>	
				<i>Gigantomonas</i>	
<i>Mastotermes</i> spp.	Parabasalia			<i>Stephanonympha</i>	[70][71][72]
		Trichonymphida	Spirotrichosomidae	<i>Leptospiromypha</i>	
				<i>Deltotrichonympha</i>	
		Cristamonadida	Lophomonadidae	<i>Koruga</i>	
		Trichomonadida	Trichomonadidae	<i>Metadevescovina</i>	
				<i>Mixotricha</i>	
				<i>Pentatrichomonoides</i>	

2.3. 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 [73]. 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 [74]. 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 [75]. 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* [76], *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*, *Byssochlamys*, *Spiromastox*, and *Malassezia*, have been defined as core microbiota in *Microcerotermes strunkii*, *Nasutitermes corniger*, and *Termes riograndensis* [77].

3. 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 1); 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 [78]. 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.

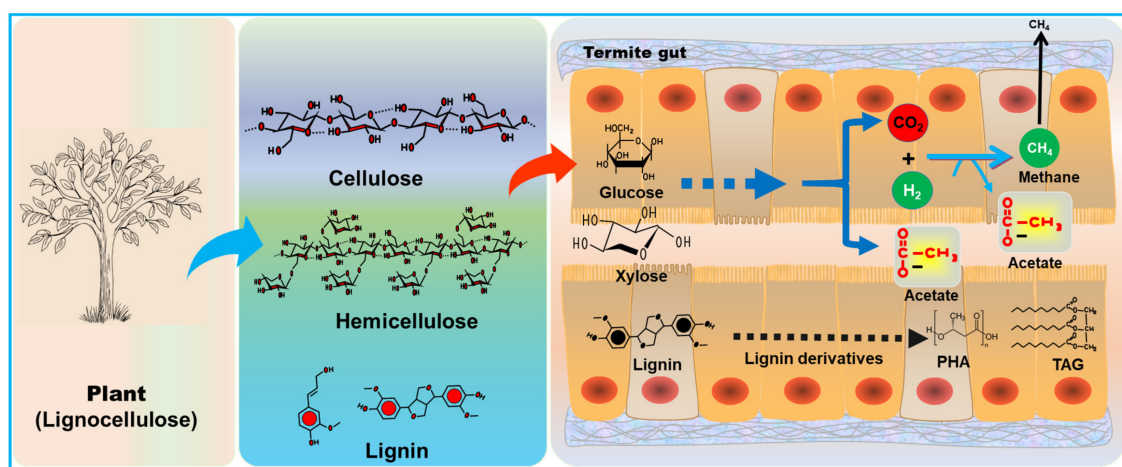


Figure 1. An overview of the metabolic pathways employed by the termite holobiome community for the digestion of lignocellulose within the gut systems. PHA: Polyhydroxyalkanoate; TAG: Triacyl glycerate.

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 [79]. Droge et al. cultured a spirochete isolated from the hindgut of a lower dry-wood termite, *Neotermes castaneus*, on different carbon sources [34]. 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 [42][80]. These spirochetes have been isolated from five species of termites, viz, *Cryptotermes cavifrons*, *Kaloterms flavicollis*, *Heterotermes tenuis*, *Neotermes mona*, and *Reticulitermes grassei*, that feed on dry-wood, indicating their coevolution with host termites [81]. Acetic acid production by the symbiotic bacteria is also reported in termites [27]. The gut symbionts, such as *Sporomusa termitida*, *Acetonema longum*, and *Clostridium mayombe*, are known to contribute 1/3 of the acetic acid for termite respiration [82]. Since *Lactococci* and *Enterococci* are well-known as cellobiose and xylose fermenters, they may be responsible for acetic acid production [83]. Hydrogen is also released by higher termites, where protists and bacterial fermentations are noted to produce molecular hydrogen (H₂) [84]. For example, numerous 16S rRNA genes isolated from the gut system of *C. orthognathus* [85] exhibit close relation to genes from the cellulolytic, hydrogen-producing bacterium, *Clostridium termitidis* [78]. Similarly, Kane and Breznak [86] 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 [82]. 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. [87] 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 [78]. 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 [88].

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) [89]. 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 [90]. 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 [91]. 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. [92] 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 [93]. 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	[94]
<i>Streptomyces</i> sp.	<i>Microcerotermes</i> species	Bioethanol	Wheat straw	[95]
<i>Bacillus</i> sp. BMP01, <i>Ochrobactrum</i> <i>oryzae</i> BMP03	<i>Cryptotermes brevis</i>	Bioethanol	CMC, Xylan, Lignin	[96]

Microorganism	Termite Host	Biofuel Type	Carbon Source	References
Bacterial symbionts	<i>Nasutitermes ephratae</i> , <i>Microcerotermes parvus</i> , <i>N. lujae</i> , <i>Termes hospes</i>	Biomethane	Wheat straw	[97]
Actinobacteria	<i>M. nervosus</i> , <i>Macrognaothermes sunteri</i> , <i>Tumulitermes pastinatio</i>	Hydrogen, Biomethane	Organic Carbon	[98]
Symbionts	<i>Reticulitermes speratus</i>	Hydrogen	Wood	[99]
<i>Treponema primitia</i>	<i>Zootermopsis angusticollis</i>	Acetate	Wood	[100]
<i>Clostridium termitidis</i> , <i>Clostridium beijerinckii</i>	<i>Nasutitermes</i> spp.	Hydrogen	Cellulose	[101]
<i>Methylocella</i> sp., and other symbionts	<i>R. speratus</i> , <i>Z. nevadensis</i> , <i>Cubitermes orthognathus</i>	Biomethane, Hydrogen	Wood	[93][102][103]
<i>Enterococcus</i> sp.	<i>R. flavipes</i>	Acetate	Lignocellulose	[104]
<i>Sporomusa termitida</i> , <i>Sporomusa</i> sp. strain TmA03	termites	Hydrogen	Lignocellulose	[41]
Microbiota	<i>Cubitermes</i> spp.	Biomethane	Lignocellulose	[105]
<i>Sporomusa aerivorans</i>	<i>Thoracotermes macrothorax</i>	Hydrogen	Lactate	[106]
<i>Sporotalea propionica</i>	<i>T. macrothorax</i>	Hydrogen	Glucose	[107]
<i>Acetonema longum</i>	<i>Pterotermes occidentis</i>	Hydrogen	Yeast extract, Rumen fluid, Resazurin	[86]
<i>Enterobacter cloacae</i> KBH3	<i>Globitermes</i> sp.	Hydrogen	Glucose	[91]
<i>Pseudotrichonympha grassii</i>	<i>C. formosanus</i>	Hydrogen	Wood cellulose	[84]
<i>Trichonympha sphaerica</i>	<i>Z. termites</i>	Hydrogen	Lignocellulose	[108]
<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	[103]
<i>Trichomitopsis termopsidis</i> , <i>Trichonympha sphaerica</i>	<i>Z. termites</i>	Hydrogen, Biomethane	Cellulose, Corncob, Cereal leaves	[109]

CMC: CarboxyMethyl Cellulose.

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