https://doi.org/10.48047/AFJBS.6.14.2024.6618-6637

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Termite Gut Microbiome and Its Role in Lignocellulose Degradation

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Article History
Volume 6, Issue 14, Aug 2024
Received:03 June 2023
Accepted : 31 July 2023
Published :22 Aug 2024
doi:10.48047/AFJBS.6.14.2024.6618-6637

Abstract

This review explores the potential of utilizing termite gut microbiota for the pretreatment of agricultural wastes. The main goal is to develop an efficient, environmentally friendly, and cost-effective method for managing lignocellulosic biomass. However, its complex and recalcitrant nature poses significant challenges to efficient biodegradation and conversion. It discusses the role of various gut compartments and the synergistic actions of resident microorganisms. Key microbial genera involved in cellulose and hemicellulose breakdown are discussed, along with the enzymatic pathways involved in plant polysaccharides. The review also examines the metabolic pathways of sugar fermentation and methanogenesis. The role of methanogens in hydrogen removal and methane production is examined, emphasizing the importance of cross-feeding interactions among microbial species. By harnessing the natural capabilities of termite associated microorganisms, this approach addresses the challenges posed by conventional pretreatment methods, contributing to sustainable agricultural waste management practices.

Key words: *Termite gut microbiome, lignocellulose degradation, cellulose, hemicellulose, methanogenesis, cross-feeding.*

Introduction

1.1 Lignocellulose as a biomass resource

In India, an estimated 85-95 million tons of paddy straw are discarded annually, primarily through burning or landfilling, due to its slow rate of degradation, disease infection potential, variable nutrient profile, and the adverse short-term effects of nitrogen immobilization on crop yield (Bhiday, 1994; Nagavallemma et al., 2006; Zeinhom et al., 2010; Pandey et al., 2009). The practice of burning agricultural residues is widespread due to its cost-effectiveness and ease, yet it releases substantial amounts of greenhouse gases such as methane, carbon

dioxide, sulfur dioxide, carbon monoxide and nitrous oxide into the atmosphere (Kausar et al., 2010). Additionally, burning produces dangerous pollutants like polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-p-dioxins (PCDDs) which are potential carcinogens with severe health implications (Gadde et al., 2009). Consequently, the efficient disposal and management of paddy straw have become critical global concerns.

The structural complexity and recalcitrance of lignocellulosic biomass, such as paddy straw, pose significant challenges to its degradation. Lignocellulose, a significant structural component of plant biomass consists of three main polymers i.e. cellulose, hemicellulose, and lignin. Rigid plant cell walls, which offer structural integrity and defence against microbial invasion, are made of these complex polysaccharides. Since it is abundant, renewable, and has the capacity to provide energy sustainably, lignocellulose is a biomass resource which is of enormous relevance. One important component, cellulose, has a highly crystalline structure that prevents it from being hydrolyzed by enzymes because of its densely packed fibres, which make enzyme accessibility difficult (Itakura *et al.*, 1997; Nakashima *et al.*, 2002). It is possible to hydrolyze cellulose to produce glucose, a fermentable sugar that can be utilised to produce biofuel. Because of its varied sugar components and glycosidic connections, hemicellulose is a heterogeneous polymer that makes full breakdown more difficult and requires a wide range of enzymes. It is essential for the flexibility and porosity of plant cell walls, serving as a filler material in between cellulose microfibrils. The structure of lignin, which encases cellulose and hemicellulose, is aromatic and extensively cross-linked, giving it structural stiffness and resistance to enzyme attack. It provides a defensive layer of phenylpropanoid units around the carbohydrate polymers. Lignin is a difficult resource to convert from biomass due to its recalcitrance (Zhao *et al.,* 2012; Sun and Cheng, 2002).

1.2 Termites and their unique ability to degrade lignocellulose

Insects, particularly termites, and their gut microbiota offer a promising alternative for the pretreatment of agricultural wastes. Termite guts harbor unique microbial communities that play essential roles in lignocellulose degradation. Unlike mammalian guts, termite guts contain fewer but highly specialized bacterial species that aid in breaking down complex plant polymers into simpler, utilizable forms (Brune and Dietrich, 2015). These microorganisms, including flagellated protists, yeasts, and bacteria, produce an array of enzymes that facilitate the digestion of cellulose, hemicellulose, and lignin (Schafer et al., 1996; Adams and Boopathy, 2005). Termites are social insects known as the “silent destroyer”. Wood-feeding termites are important decomposers with mouths

perfectly designed for tearing both dead and living wood apart. These organisms obtain essential nutrients by breaking down cellulose, a fibrous component present in dead wood and plant debris. They are similar in appearance to ants and are commonly called “white ants”. Termites are ecologically very significant because of their ability to degrade lignocellulose, and this ability depends on the gut microbial community of the insect (Ohkuma 2003). Termites are reported to take a major part in recycling a mixture of cellulose, hemicellulose and lignin referred as lignocellulosic biomass (Upadhyaya *et al*., 2012). When ingesting lignocellulose, termites effectively break down a significant portion of the cellulose (74-99%) and hemicellulose (65-87%) content (Ohkuma 2003). Termites help in turnover and mineralization of wood and other materials containing cellulose and hemicellulose (Wenzel *et al.;* 2002). Cellulose degradation is significantly influenced by the composition of the gut microflora, with symbiotic flagellates playing a crucial role in this process. Adapted to their ecological niche, termites independently regulate moisture within their colonies, enabling them to collect and utilize significant amounts of litter irrespective of the surrounding temperature. A diverse gut microbiota facilitates the digestion of wood, bark, and straw by termite species through the production of endogenous enzymes (Jouquet *et al.,* 2011). Recent studies have shown that termites have the ability to produce their own cellulases whether they harbour protists or not (Tokuda *et al.,* 2012). Lower and higher termites possess gut cellulases, enzymes that break down cellulose, a major component of their woody diet. Cellulases account for roughly 8% of global industrial enzyme demand (Behera et al., 2016), finding applications in various sectors, particularly those focused on agricultural and industrial waste treatment (Kuhad *et al*., 2011). Scientists have been captivated by termites' remarkable capacity to break down lignocellulosic biomass for many years. Their intestines are inhabited by a variety of highly specialized microbial communities that are largely responsible for their incredible ability. These microorganisms, which include bacteria, archaea, and protozoa, cooperate to convert complex plant elements into simpler substances that termites can consume. Microorganisms can exist as a colony of cells or as a single cell, and they make up the enormous and varied microbial world. The earliest life forms on Earth were thought to be single-celled microbes (Schopf, 2006). The rate of evolution of microorganisms is often quite rapid. Bacteria are ubiquitous in nature, meaning they can be found in nearly every type of habitat found in nature. They have been found in the highest mountain peaks to the deepest ocean depths, and they can be found in water, soil, and air surrounding us. They can also be found on and in the food we consume, inside human bodies, and even in extreme environments like extremely salted water bodies, rocks up to seven kilometers below the surface of the Earth, boiling hot springs, and snow-covered fields (Szewzyk et al., 1994).

2. Termite Gut Structure and Function

Termites, classified into upper and lower termites, are a complex group of different species that belong to the order Isoptera. Prokaryotes and protists are abundant in lower termites' digestive tracts. For lower termites to break down cellulose in their guts with such high efficiency, cellulolytic protists must be present. Unlike lower termites, whose guts are inhabited by protists, higher termites possess a distinct gut microbiome devoid of these

single-celled organisms (Ohkuma, 2003). Within the termite gut, protists break down wood and cellulose particles into acetate, an essential molecule absorbed by the termite as both an energy and carbon source, establishing a mutually beneficial symbiosis (Ohkuma, 2003). The termite gut exhibits a structured microenvironment characterized by distinct zones with variations in metabolic activities and microbial communities (Kohler et al., 2012).

Foregut, midgut, and hindgut are the three parts of termite gut. The crop and the gizzard (proventriculus) comprise the foregut, which is mostly used for food ingestion, storage, and mechanical processing. Termites swallow lignocellulosic material, which is ground into tiny particles by their powerful mandibles, which increases the surface area available for enzymatic action. The salivary glands release cellulases here, which initiate the breakdown of cellulose, albeit with modest enzymatic activity. The enzymatic breakdown of lignocellulosic polymers and the absorption of nutrients are mostly carried out in the midgut. Termites lead to the formation of simpler sugars by breaking down of cellulose and hemicellulose with the help of their own cellulases and hemicellulases. The complex carbohydrates are broken down into monosaccharides and oligosaccharides by the enzymes in the midgut. As the largest organ hindgut harbours numerous microorganisms. The termite gut has microbes from all three major groups of living things (Archaea, Bacteria, and Eukaryota) (Ni and Tokuda, 2013). The majority of the digestive processes are carried out by a dense and varied community of symbiotic bacteria found in the hindgut, making it the most important compartment for the degradation of lignocellulose. Cellulases, which break down cellulose into cellobiose and glucose, are produced by bacteria like *Clostridium* and *Cellulomonas.* Hemicellulases are produced by bacteria such as *Bacillus* and *Paenibacillus,* which hydrolyze hemicellulose into xylose and other sugars. Protozoa, such those in the genus *Treponema*, breaks down the cellulose and are home to endosymbiotic bacteria (Leadbetter *et al.,* 1999). Methanogenic archaea reduce hydrogen buildup and improve fermentation efficiency by converting carbon dioxide and hydrogen generated during fermentation into methane. While lignin is mostly resistant to degradation, some bacteria and fungi can partially break it down, making cellulose and hemicellulose more accessible. Higher termites have more complex and advanced hindgut structures compared to lower termites. The foregut, midgut, and hindgut of termites are three discrete parts of the gut that each support various microbial communities and offer environmental conditions that facilitate the breakdown of lignocellulosic material (Brune and Dietrich, 2015). The basic constituents of lignocellulosic biomass, cellulose, hemicellulose, and lignin are broken down by a variety of enzymes produced by a broad array of bacteria that thrive in the extremely anaerobic environment of the hindgut (Brune, 2014).

3. The Termite Gut Microbiome

The microbiome constitutes the entire genetic composition of microorganisms, including bacteria, fungi, protozoa, and viruses, inhabiting the human body. The collective gene count of an individual's microbiome exceeds that of the human genome by a factor of 200. A microbiota represents a microbial community comprising commensal, symbiotic, and pathogenic organisms that coexist within and on all multicellular organisms.

Encompassing bacteria, archaea, protists, fungi, and viruses, the microbiota plays a critical role in nutrition, immunity, and behavioural regulation. Microorganisms form the foundation of the microbiota found within and on all multicellular life, with the most substantial bacterial populations residing in the gut. The gut microflora or gastrointestinal microbiota is a complex ecosystem of microorganisms inhabiting the digestive tracts of humans and other animals, including insects. Intestinal microorganisms are essential for host nutrition, physiology, behaviour, development, and defence against pathogens. The diversity of the insect gut microbial community is crucial for host survival by facilitating cellulose degradation and providing essential sugars. Insect digestive systems harbour a diverse microbial population composed of resident and transient bacteria, fungi, protozoa, and archaea. These microorganisms contribute to various insect physiological functions, such as food digestion, nutrient acquisition, nitrogen fixation, and pheromone production (Lilburn et al., 2001; Dillon and Dillon, 2004). The symbiotic association between wood-feeding termites and their gut bacteria has been extensively studied (Breznak, 1982; Ohkuma, 2003).

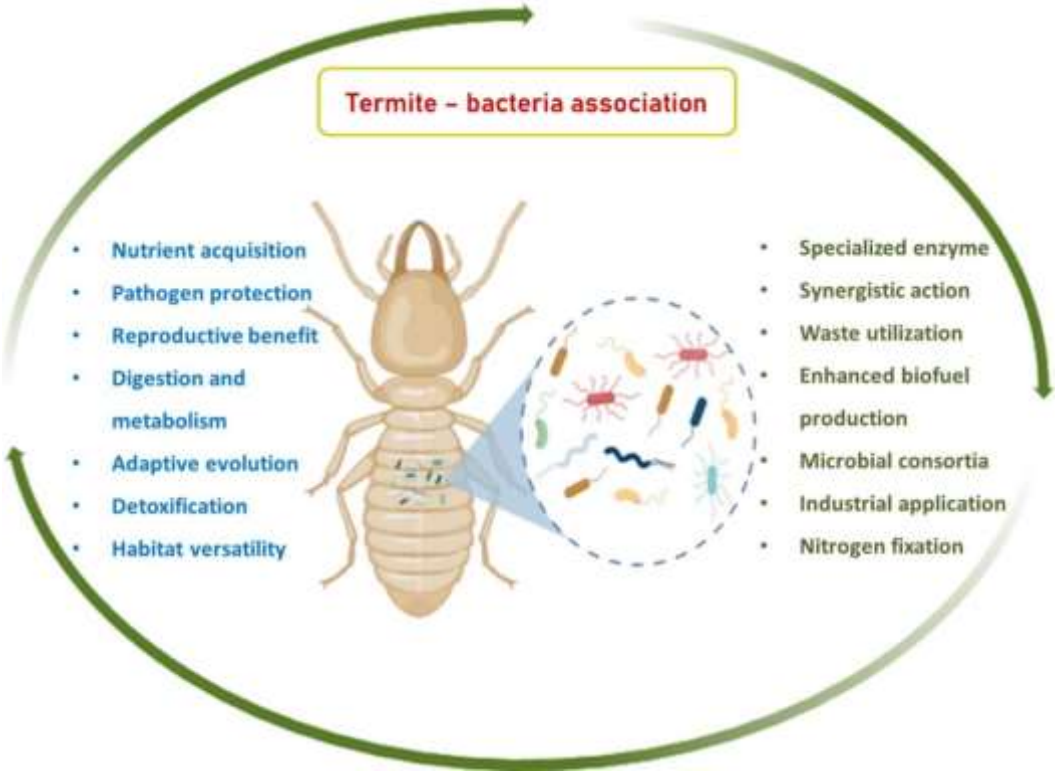

Figure 1: Diagram illustrating the relationship between microorganism and termites.

3.1 Microbial Diversity in Termite Gut

Plant cell walls are primarily composed of lignocellulose, which is broken down by a complex variety of bacteria found in termite guts. Several microbes have been isolated from termite guts, known for their unique ability to degrade lignocellulosic materials. Some ciliates, anaerobic fungi and anaerobic bacteria belonging to the genera *Bacteroides, Butyrivibrio, Fibrobacter* and *Ruminococcus* accomplish the digestion of cellulose in the rumen. *Bacteroides* sp. also plays a role in the degradation of hemicellulose and other polysaccharides. *Bacteroides*

species produce a variety of enzymes, including cellulases and hemicellulases, which break down cellulose and hemicellulose into simpler sugars. They secrete enzymes like β-glucosidase, xylanase, and mannanase, which help in the hydrolysis of lignocellulose components (Thomas et al., 2011). Through co-metabolism and cross-feeding, *Bacteroides* interact with other bacteria in the gut to increase the effectiveness of lignocellulose breakdown (Morris et al., 2019).

The cellulolytic enzymes found in *Butyrivibrio* species enable the breakdown of cellulose into glucose, which can subsequently undergo fermentation to yield butyrate and additional short-chain fatty acids (SCFAs). They generate a variety of enzymes that attack the internal and terminal linkages of cellulose, such as endoglucanases and exoglucanases (Koike and Kobayashi, 2001). According to Kelly et al. (2010), *Butyrivibrio* species aid in the breakdown of plant fibres and improve the availability of nutrients for the host by contributing to the overall fermentation process in the termite gut.

The *Fibrobacter succinogenes* efficiently helps in cellulose degradation and often found in the hindgut of termites. *Fibrobacter* species are extremely specialised in breaking down cellulose and turning it into soluble sugars, especially *Fibrobacter succinogenes* (Suen et al., 2011). These bacteria effectively hydrolyze cellulose to glucose and other monosaccharides by producing a diverse variety of cellulases and cellulosomes (Pedroza and Iyavoo 2023). According to Warnecke et al. (2007), *Fibrobacter* species play a critical role in the termite gut ecosystem by aiding in the breakdown of plant matter and sustaining the host's nutritional requirements.

Many cellulases and hemicellulases are produced by *Ruminococcus* species, including *Ruminococcus albus* and *Flavefaciens*, which are known for their strong cellulolytic activity (Flint et al., 2008). By concentrating on several polysaccharide components at the same time, these bacteria combine to form multi-enzyme complexes called cellulosomes, which effectively break down plant cell walls (Ding et al., 2001). In the termite gut, *Ruminococcus* species work in combination with other fermentative and cellulolytic bacteria to improve the overall efficiency of lignocellulose degradation (Weimer, 1998).

Several bacteria in the termites' gut form an intricate ecology that is essential to the breakdown of lignocellulose, a significant source of nourishment for them. Moreover, certain *Clostridium sp*. is known for producing potent cellulases and playing a significant role in the breakdown of cellulose. These anaerobic bacteria create cellulases and other enzymes that can degrade lignocellulosic materials. Termites rely on them to digest cellulose into volatile fatty acids (VFAs), which are essential for their energy.

There are also reports on *Sporocytophaga sp.,* capable of degrading cellulose and contributing to the overall digestion process. Certain ligninolytic bacteria such as *Actinomyces sp.* and *Streptomyces sp*. are involved in the breakdown of lignin and complex aromatic compounds and are known for producing a wide range of lignin-degrading enzymes respectively. Isolates with similarity to *Cellulomonas cartae* were isolated from six other termite species. A third group of cellulolytic microorganisms in the termite gut, besides flagellates and bacteria, may be characterized by yeast (Prillinger *et al.,* 1996). Some of the yeast isolates in the termite gut were able to

break down cellulose and some of them were able to produce hemicellulases (Wenzel *et al.,* 2002). The gut of wood-eating termite contains abundant and diverse Spirochetes which are both free-swimming in the gut as well as protist episymbionts. The cellulolytic bacteria exhibited carboxymethyl-cellulose (CMC) degradation on the basis of decolorization of CMC agar medium using Congo red as a colour indicator (Khianngam *et al*, 2014). Studies have reported that the gram positive bacteria belonged to *Bacillus* which produces a variety of enzymes that help in the degradation of plant material. Extracellular enzymes like cellulases and xylanases, which help break down lignocellulosic materials in termites' guts, are produced by *Bacillus* species. Reports of *Paenibacillus* suggests that they have the potential of producing a variety of enzymes that break down hemicellulose and other plant polysaccharides and *Lysinibacillus* on the basis of phenotypic characteristics and 16S rRNA gene sequence analysis (Khianngam *et al*, 2014). In addition, there are also certain general lignocellulose degrading bacteria which are versatile in degrading various components of lignocellulose, including cellulose and hemicellulose such as *Enterobacter sp.*, *Pseudomonas sp.*, *Acinetobacter sp.,* etc. Several studies have also reported that there are many symbiotic bacteria which assist in cellulose digestion and often form symbiotic relationships with termites such as *Treponema sp.*, *Desulfovibrio sp. etc.* The *Desulfovibrio sp.* is sulfate reducing bacteria that participate in lignocellulose degradation in anoxic conditions and aids in the sulphur cycle in the termite gut as well as in the mineralization of organic materials. Certain by-products such as methane are produced by methanogenic bacteria *Methanobrevibacter sp*. in the gut of termites in the degradation of lignocellulose. They also aid in the last stages of fermentation by producing methane from hydrogen and carbon dioxide. This maintains the gut environment balanced and inhibits hydrogen accumulation that could hinder cellulolytic bacteria.

Table 1: Certain microorganisms isolated from gut of different termite species

MICROORGANISMS	**TERMITE SPECIES**	**REFERENCE**
Bacillus brevis	*Zootermopsis angusticollis*	Wenzel et al (2002)
Bacillus cereus	*Nasutitermes nigriceps* *Reticulitermes hesperus* *Reticulitermes santonensis* *Neotermes castaneus*	Kuhnigk and Konig (1997); Schafer et al (1996)
Bacillus coagulans	*Mastotermes darwiniensis*	Schafer et al (1996)
Bacillus firmus	*Reticulitermes santonensis*	Kuhnigk et al (1996)
Bacillus licheniformis	*Reticulitermes santonensis*	Kuhnigk and Konig (1997); Schafer et al (1996)
Bacillus megaterium	*Mastotermes darwiniensis* *Zootermopsis angusticollis*	Kuhnigk (1996); Mannesmann and Piechowski (1989); Wenzel et al (2002)

Bacillus sp	*Schedorhinotermes intermedius* *Coptotermes acinaciformis* *Coptotermes formosanus* *Heterotermes indicola*	Eutick et al (1978); Kuhnigk et al (1996); Mannesmann and Piechowski (1989)
Bacillus sphaericus	*Odontotermes distans* *Odontotermes obesus* *Reticulitermes santonensis* *Zootermopsis angusticollis*	Kuhnigk et al (1996); Kuhnigk and Konig (1997); Schafer et al (1996)
Bacillus subtilis	*Reticulitermes santonensis*	Kuhnigk and Konig (1997); Schafer et al. (1996)
Paenibacillus macerans	*Mastotermes darwiniensis*	Schafer et al. (1996)
Paenibacillus sp.	*Zootermopsis angusticollis*	Wenzel et al. (2002)
Enterococcus faecalis	*Mastotermes darweniensis* *Reticulitermes flavipes*	Bauer et al. (2000);
Lactobacillus sp.	*Reticulitermes flavipes*	Schultz and Breznak (1978)
Staphylococcus sp.	*Nasutitermes exitiosus* *Nasutitermes graveolus* *Nasutitermes walker*	Eutick et al. (1978)
Streptococcus sp. *Streptococcus* sp.	*Nasutitermes exitiosus* *Mastotermes darweniensis* *Reticulitermes flavipes* *Coptotermes lacteus* *Cryptotermes primus* *Heterotermes ferox*	Eutick et al. (1978) Kuhnigk et al (1996) Potrikus and Breznak (1980)

Pseudomonas sp.	*Coptotermes formosanus* *Nasutitermes nigriceps* *Reticulitermes lucifugus* *Odontotermes distans*	Kuhnigk et al (1996) Mannesmann and Piechowski (1989)
	Odontotermes obesus	

These bacteria work together in the complex microbial ecosystem of the termite gut, contributing to the efficient breakdown of lignocellulosic materials. Their ability to produce a wide range of degrading enzymes makes them valuable for biotechnological applications, including biofuel production and waste management.

3.2 Functional Roles

Gut bacteria offer various benefits to their hosts, and the insect gut provides a unique environment for microbial colonization. Compared to mammalian digestive systems, most insect guts harbor fewer microbial species, although some possess substantial and specialized gut bacterial communities. The factors influencing microbial community composition within insect digestive tracts vary widely, encompassing morphological and physicochemical conditions. Social insects, such as termites, facilitate the exchange of gut bacteria, often resulting in distinct and stable gut microbial communities with specialized roles in nutrition and protection. Insects represent the most abundant and diverse animal group in terms of ecological distribution and biomass. Their evolutionary success is partly attributed to their associations with beneficial microorganisms, which enhance nutrient acquisition from poor diets, aid in digesting complex food components, provide defense against predators, parasites, and pathogens, influence communication, and impact disease transmission efficiency. Microbial communities are particularly prominent in the digestive tracts of all animals, where they play a pivotal role in shaping the diverse lifestyles of insect hosts. Microorganisms are integral to human culture and well-being, contributing to food fermentation, sewage treatment, fuel production, and the creation of enzymes and other bioactive compounds. They serve as essential research tools in biology and have been exploited for biological warfare and bioterrorism (Cenciarelli et al., 2013). The impact of gut microorganisms on insect function holds significant relevance across various fields, including medicine, agriculture, and ecology. Insects cause substantial agricultural damage while also pollinating many food crops, and associated microorganisms can influence their interactions with plants.

4. Mechanisms of Lignocellulose Degradation

4.1. Enzymatic Breakdown

A complicated interplay of several enzymes produced by termites and their symbiotic gut bacteria facilitates the

enzymatic breakdown of lignocellulose (Adsul *et al.,* 2020; Zhao *et al.,* 2021). The linear polymer of β-1,4-linked glucose units is called cellulose. A group of cellulases break it down enzymatically, working together to transform cellulose into glucose. (Sukumaran *et al.,* 2005; Singhania *et al.,* 2010). Endoglucanases form new chain ends and decrease the degree of polymerization by cleaving internal β-1,4-glycosidic linkages inside the cellulose chain. Exoglucanases release cellobiose, a disaccharide of glucose, by acting on the reducing and non-reducing ends of cellulose strands. Cellobiose and other short cello-oligosaccharides are hydrolyzed into glucose monomers by β-glucosidases ((Adsul *et al.,* 2020; Agarwal *et al.,* 2013). Moreover, hemicellulose is a heterogeneous polymer made up of different monomers of sugar such as xylose, mannose, galactose, and arabinose. Different kinds of enzymes are required for the degradation of hemicellulose. These enzymes break the β-1,4-glycosidic linkages which comprise the xylan backbone, which is the main building block of hemicellulose. Hydrolyzing xylo-oligosaccharides into xylose monomers is accomplished by β-xylosidases. The hemicellulose's mannan backbone is broken down by mannanases, yielding mannose. The enzymes arabinanases and α-L-arabinofuranosidases break down arabinan and arabinoxylan structures, releasing arabinose (Patel *et al.,* 2020). Additionally, encasing cellulose and hemicellulose, lignin is a complex aromatic polymer which provides structural rigidity and resistance to deterioration. In order to obtain the carbohydrates contained in lignocellulose, it has to be broken down. The oxidative cleavage of lignin structures is catalysed by lignin peroxidases, which produce free radicals that cause lignin depolymerization. Mn (II) is oxidised to Mn (III) by manganese peroxidases, which oxidises lignin compounds and promotes lignin degradation. Laccases are copper-containing oxidases that catalyse lignin modification and depolymerization by oxidising phenolic chemicals in lignin (Patel *et al.,* 2020).

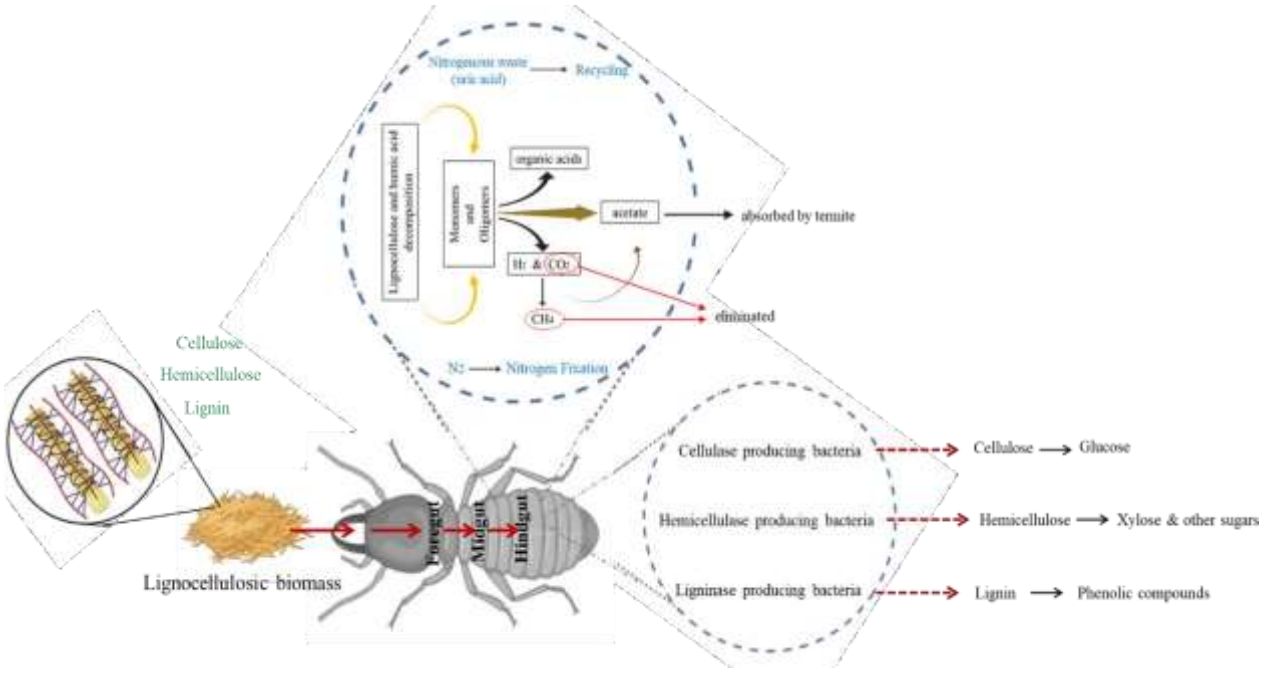

Figure 2: Overview of uptake and breakdown of lignocellulose by termite gut microbiome

4.2. Fermentation and Methanogenesis

The termite gut breaks down lignocellulosic material into a variety of simple sugars. The gut microbiota then ferments these sugars to produce gases, short-chain fatty acids (SCFAs), and other metabolites. These fermentation processes are essential for transforming cellulose and hemicellulose breakdown products into molecules that are rich in energy which termites can utilise.

Fermentation requires glucose and other hexose sugars that are produced from the breakdown of cellulose and hemicellulose. Fructose-1,6-bisphosphate is generated when phosphorylation occurs in glucose and other hexose carbohydrates. Subsequently breaking down of fructose-1,6-bisphosphate leads to the production of two molecules with three carbons each: glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. These intermediates are then processed further to yield pyruvate, while also producing ATP and NADH. The rapid energy source (ATP) and reducing equivalents (NADH) that glycolysis produces are essential for other metabolic processes, which makes it a crucial activity. Pyruvate, ATP, and NADH are the fermentation processes' end products. Since the key intermediary in a number of fermentation routes is pyruvate, which is the final by-product of glycolysis, therefore, pyruvate undergoes a number of conversion processes into distinct metabolites in the anaerobic termite hindgut environment. Further, the pyruvate is converted to acetyl-coenzyme A, which is converted into acetate by acetyl-CoA pathway where the key enzymes pyruvate-formate lyase and acetate kinase accelerates the production of acetate. As a result, acetate serves as a major short-chain fatty acid which is absorbed and utilized by termites for energy. In addition to acetate, butyrate is another short-chain fatty acid that is beneficial for termite in maintaining the gut health and also provides energy for their regular activities. Thus, pyruvate is converted into butyryl-CoA which is followed by conversion into butyrate by the enzyme butyryl-CoA dehydrogenase. Simultaneously, succinate and lactate are produced by the conversion of the pyruvate into propionate and lactate respectively. The adenosylcobalamin-dependent enzyme methylmalonyl-CoA muatse is responsible for the production of propionate which serves as a significant energy source and plays a role in different biosynthetic pathways, whereas lactate dehydrogenase act as a catalyst in lactate production by homolactic fermentation and this molecule can be further metabolized by other gut microbes into short-chain fatty acids or gases (Brune, 2014; Leadbetter *et al.*, 1996; Breznak and Switzer, 1986; Pope *et al.*, 2012).

Methanogenesis is the process by which methanogenic archaea produce methane (CH_4) biologically. In anaerobic conditions like the termite gut, where it aids in the removal of surplus hydrogen and other fermentation byproducts, this procedure is crucial. Methanogenesis can occur through three primary pathways, namely hydrogenotrophic, acetoclastic, and methylotrophic, which vary depending on the substrates that are utilised.

In hydrogenotrophic pathway, hydrogen and carbondioxide are utilized in the production of methane. Initially, the enzyme formate dehydrogenase helps in the reduction of carbondioxide to formate (HCOO-). Further, formate

is reduced to methylene tetrahydromethnopterin (CH_2H_4-MPT), and this methylene group is reduced to a methyl group (CH_3H_4-MPT) through several intermediates. Methyl-coenzyme M reductase (MCR) reduce the methyl group to methane. In order to complete this overall process, Coenzyme M (CoM) and Coenzyme B (CoB) are regenerated. The acetate formed during the fermentation process is further converted to methane and carbondioxide through acetoclastic methanogenesis pathway. The enzyme acetyl-CoA synthetase activates acetate to acetyl-CoA. Following activation, the later form is cleaved into a methyl group and carbon monoxide. In addition to replenishing cofactors, methyl-coenzyme M reductase oxidises carbon monoxide to carbon dioxide and reduces the methyl group to methane. Moreover, the methylated compounds such as methanol and methylamines are reduced to methane via methylotrophic methanogenesis. Initially, the methylated compounds are activated to form methylated coenzyme M (CH_3–CoM). The methyl- coenzyme M reductase reduces the methyl group to methane (Leadbetter *et al.,* 1996; Thaeur *et al.,* 2008; Liu and Whitman, 2008; Brune, 2014).

4.3. Synergistic Interactions

The metabolic interdependence between distinct microbial species, whereby the byproducts of one organism serve as substrates for another, is known as cross-feeding or syntrophic interactions. This event is essential for the effective breakdown of lignocellulose in the termite gut as well as for the overall stability of the microbial ecology. For instance, methanogens and acetogens use the hydrogen generated by cellulolytic bacteria. Methanogens and acetogenic bacteria interact during syntrophic acetate oxidation to transform acetate into CO_2 and methane. The gut's diverse microbial species are spatially close to one another, facilitating the effective transmission of metabolic intermediates. An illustration of this could be the assembly of methanogens that consume hydrogen and bacteria that produce hydrogen in the gut microenvironment. Thus cross-feeding ensures that lignocellulosic materials are completely broken down and utilised, resulting in the highest possible extraction of energy. By keeping inhibitory byproducts from building up, syntrophic interactions maintain the stability of the microbial community. Termite growth and survival are supported by the efficient harvesting of energy through the generation and utilisation of short-chain fatty acids and methane (Brune, 2014; Leadbetter *et al.,* 1996; Breznak and Switzer, 1986; Pope *et al.,* 2012).

Enzyme coordination is necessary for the effective breakdown of lignocellulose, and this coordination is frequently made possible by the mutualistic relationships among several microbial species in termites' digestive tracts. Cellulases are better able to reach cellulose fibres when hemicellulose is broken down by hemicellulases, exposing them. Termites and their gut bacteria have a very synergistic relationship. Termites supply lignocellulosic material continuously to establish an ideal anaerobic environment for microbiological activities. In exchange, the bacteria break down the complex plant polymers to provide the termites with vital nutrients and energy (Brune and Dietrich, 2015). The survival of the termite and its effectiveness in decomposing biomass depend on this mutualistic interaction. Studies have reported that the symbiotic relationship between phylogenetically lower termites and the unicellular organisms colonizing their hindguts is critical. The protists

comprising the termite gut have the ability to ferment the cellulose into acetate, which can be used as an energy source by the termite (Breznak and Brune, 1994). The flagellated protists, yeast, and bacteria present in the digestive tract of termite produce enzymes. Due to this enzymatic activity, the termites have the capability to digest wood containing high fiber. Diverse aerobic and facultatively anaerobic bacteria and yeasts were cultured from termites using xylan, arabinogalactan, and carboxymethyl cellulose as growth substrates. Gram-positive bacterial isolates were classified within the genera Bacillus, Paenibacillus, Streptomyces, or the Actinobacteria, while Gram-negative isolates were assigned to Pseudomonas, Acinetobacter, Ochrobactrum, and the Enterobacteriaceae (Schafer et al., 1996). Hungate (1946) isolated an anaerobic, cellulolytic Actinomycete (M. propionici) from the gut of Amitermes minimus but deemed its in-situ significance limited. Four facultative anaerobic, Gram-negative enteric bacteria were recovered from the hindgut of the Formosan subterranean termite. Identified as Serratia marcescens, Enterobacter aerogenes, Enterobacter cloacae, and Citrobacter farmer through BIOLOG assay and fatty acid methyl ester analysis, these isolates were characterized further (Adams and Boopathy, 2005). The most common bacteria found in termites are *Clostridia* and *Bacteroidia*, with different species and strains being present in different termite species and even in different gut compartments (Warnecke *et al.,* 2007). Methanogenic archaea are also present in termite guts, aiding in the breakdown of hydrogen and carbon dioxide into methane, thus helping in the overall energy metabolism of termites (Leadbetter *et al.,* 1999).

5. Challenges in Degradation

5.1. Structural Complexity and Recalcitrance

Complex Structure: Cellulose has a highly crystalline structure, which makes it resistant to enzymatic hydrolysis (Zhu *et al.,* 2008). The tight packing of cellulose fibers hinders enzyme accessibility. Hemicellulose is a heterogeneous polymer composed of various sugars, which complicates the enzymatic breakdown process. The variety of glycosidic linkages requires a broad spectrum of enzymes for complete degradation (Saha, 2003).

Lignin Encapsulation: Lignin surrounds cellulose and hemicellulose, providing structural rigidity and protecting these polysaccharides from enzymatic attack. The aromatic and highly cross-linked structure of lignin makes it extremely resistant to degradation (Chundawat et al., 2011).

5.2. Inefficiencies in Conventional Pretreatment Methods

Methods such as grinding, milling, and thermal treatments require high energy inputs, making them economically impractical for large-scale operations (Mosier et al., 2005).

Chemical Pretreatment: Chemical treatments using acids, alkalis, or solvents can leave behind toxic residues that inhibit microbial activity and require additional processing steps to neutralize (Hendriks and Zeeman, 2009). The use of harsh chemicals poses environmental risks and necessitates careful handling and disposal, increasing

operational costs.

Biological Pretreatment: Biological pretreatment using fungi or other microorganisms is often slow, taking weeks or months to achieve significant degradation. This extended time frame is not feasible for industrial applications (Sanchez, 2009). Biological methods may not fully break down lignin, leading to incomplete hydrolysis of cellulose and hemicellulose (Wan and Li, 2012).

5.3. Enzymatic Hydrolysis Challenges

The production and purification of cellulolytic and hemicellulolytic enzymes are expensive, making the process economically unviable for large-scale applications (Singhania et al., 2013). By-products such as glucose, cellobiose, and lignin derivatives can inhibit enzyme activity, reducing the overall efficiency of hydrolysis (Xiao et al., 2004).

Synergistic Action Requirement: Effective degradation of lignocellulosic biomass requires a complex mixture of enzymes working synergistically. Coordinating the production and activity of these enzyme mixtures is challenging (Bayer et al., 2010).

Conclusion

The exploration of termite gut microbiota as a pretreatment method for agricultural wastes presents a promising avenue for addressing the challenges associated with lignocellulosic biomass degradation. Termites and their symbiotic gut microorganisms have evolved highly efficient systems for breaking down complex plant polymers, offering a natural and environmentally friendly solution to the disposal and management of agricultural residues. By harnessing the capabilities of termite-associated microorganisms, this approach aims to overcome the limitations of conventional pretreatment methods, such as high energy consumption, toxic residues, and incomplete hydrolysis. Termite gut microbiota, including bacteria, archaea, and protists, produce a diverse array of enzymes capable of degrading cellulose, hemicellulose, and lignin. The mutualistic relationship between termites and their gut microorganisms is crucial for the efficient breakdown of lignocellulosic materials, providing the termites with essential nutrients and energy while facilitating the turnover and mineralization of plant biomass. The unique microbial communities in termite guts offer valuable insights into the development of biotechnological applications for biofuel production, waste management, and sustainable agricultural practices.

Future research should focus on isolating and characterizing the specific enzymes and microorganisms involved in lignocellulose degradation within termite guts. Additionally, the optimization of microbial consortia and the development of scalable processes for the pretreatment of agricultural wastes will be essential for translating these findings into practical applications. By leveraging the natural capabilities of termite gut microbiota, we can develop innovative and sustainable solutions for the effective management of agricultural residues, contributing to environmental conservation and the advancement of green technologies.

References

1. Adams, L., and Boopathy, R. (2005). Isolation and characterization of enteric bacteria from the hindgut of Formosan termite. *Bioresource Technology*, 96(14), 1592-1598.
2. Adsul, M., Sandhu, S. K., Singhania, R. R., Gupta, R., Puri, S. K., and Mathur, A. (2020). Designing a cellulolytic enzyme cocktail for the efficient and economical conversion of lignocellulosic biomass to biofuels. *Enzyme and Microbial Technology*, 133, 109442.
3. Agrawal, R., Satlewal, A., and Verma, A. K. (2017). Utilization of Citrus sinensis waste for the production of β-glucosidase by solid-state fermentation using a Bacillus subtilis mutant. *Environmental Engineering and Management Journal*, 16(7).
4. Bauer, S., Tholen, A., Overmann, J., and Brune, A. (2000). Characterization of abundance and diversity of lactic acid bacteria in the hindgut of wood-and soil-feeding termites by molecular and culture-dependent techniques. *Archives of Microbiology*, 173, 126-137.
5. Bayer, E. A., Lamed, R., White, B. A., Ding, S. Y., and Himmel, M. E. (2010). Conversion of agricultural residues to bioethanol: the roles of cellulases and cellulosomes. *Biofuels from Agricultural Wastes and Byproducts*, 67-96.
6. Behera, B. C., Mishra, R. R., Singh, S. K., Dutta, S. K., and Thatoi, H. (2016). Cellulase from Bacillus licheniformis and Brucella sp. isolated from mangrove soils of Mahanadi River delta, Odisha, India. *Biocatalysis and Biotransformation*, 34(1), 44-53.
7. Bhiday, M. R. (1994). Earthworms in agriculture. *Indian Farming* 43: 31-34.
8. Breznak, J. A., and Brune, A. (1994). Role of microorganisms in the digestion of lignocellulose by termites. *Annual Review of Entomology*, 39(1), 453-487.
9. Breznak, J. A., and Switzer, J. M. (1986). Acetate synthesis from H_2 plus CO_2 by termite gut microbes. *Applied and Environmental Microbiology*, 52(4), 623-630.
10. Brune, A. (2014). Symbiotic digestion of lignocellulose in termite guts. Nature *Reviews Microbiology*, 12(3), 168-180.
11. Brune, A., and Dietrich, C. (2015). The gut microbiota of termites: digesting the diversity in the light of ecology and evolution. *Annual review of microbiology*, 69(1), 145-166.
12. Cenciarelli, O., Rea, S., Carestia, M.C., D'Amico, F., Malizia, A., Bellecci, C., GAUDIO, P., Gucciardino, A. and Fiorito, R. (2013). Biological Weapons and Bio-Terrorism: a review of History and Biological Agents. *International Journal of Intelligent Defence Support Systems*, 6(2), 111-129.
13. Chundawat, S. P., Beckham, G. T., Himmel, M. E., and Dale, B. E. (2011). Deconstruction of lignocellulosic biomass to fuels and chemicals. *Annual Review of Chemical and Biomolecular Engineering*, 2(1), 121-145.
14. Dillon, R. J., and Dillon, V. M. (2004). The gut bacteria of insects: nonpathogenic interactions. *Annual Reviews in Entomology*, 49(1), 71-92.

15. Ding, S.Y., Rincon, M.T., Lamed, R., Martin, J.C., McCrae, S.I., Aurilia, V., Shoham, Y., Bayer, E.A. and Flint, H.J. (2001). Cellulosomal scaffoldin-like proteins from Ruminococcus flavefaciens. *Journal of Bacteriology*, 183(6), 1945-1953.
16. El Alfy, Z., Elhadary, R., and Elashry, A. (2010). Integrating GIS and MCDM to deal with landfill site selection. *International Journal of Engineering and Technology*, 10(6), 32-42.
17. Eutick, M. L., O'brien, R. W., and Slaytor, M. (1978). Bacteria from the gut of Australian termites. *Applied and Environmental Microbiology*, 35(5), 823-828.
18. Flint, H. J., Bayer, E. A., Rincon, M. T., Lamed, R., and White, B. A. (2008). Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nature Reviews Microbiology,* 6(2), 121-131.
19. Gadde, B., Bonnet, S., Menke, C., and Garivait, S. (2009). Air pollutant emissions from rice straw open field burning in India, Thailand and the Philippines. *Environmental Pollution,* 157(5), 1554-1558.
20. Hendriks, A. T. W. M., and Zeeman, G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology*, 100(1), 10-18.
21. Hungate, R. E. (1946). Studies on Cellulose Fermentation: II. An Anaerobic Cellulose-decomposing Actinomycete, Micromonospora propionici, N. Sp. *Journal of Bacteriology*, 51(1), 51-56.
22. Itakura, S., Tanaka, H., and Enoki, A. (1997). Distribution of cellulases, glucose and related substances in the body of Coptotermes formosanus. *Material und Organismen*, 31(1), 17-29.
23. Jouquet, P., Traoré, S., Choosai, C., Hartmann, C., and Bignell, D. (2011). Influence of termites on ecosystem functioning. Ecosystem services provided by termites. *European Journal of Soil Biology,* 47(4), 215-222.
24. Kausar, H., Sariah, M., Saud, H. M., Alam, M. Z., and Ismail, M. R. (2010). Development of compatible lignocellulolytic fungal consortium for rapid composting of rice straw. *International Biodeterioration and Biodegradation*, 64(7), 594-600.
25. Kelly, W. J., Leahy, S. C., Altermann, E., Yeoman, C. J., Dunne, J. C., Kong, Z., Pacheco, D.M., Li, D., Noel, S.J., Moon, C.D. and Cookson, A.L., and Attwood, G. T. (2010). The glycobiome of the rumen bacterium Butyrivibrio proteoclasticus B316T highlights adaptation to a polysaccharide-rich environment. *Plos One,* 5(8), 11942.
26. Khianngam, S., Pootaeng-on, Y., Techakriengkrai, T., and Tanasupawat, S. (2014). Screening and identification of cellulase producing bacteria isolated from oil palm meal. *Journal of Applied Pharmaceutical Science,* 4(4), 90-96.
27. Köhler, T., Dietrich, C., Scheffrahn, R. H., and Brune, A. (2012). High-resolution analysis of gut environment and bacterial microbiota reveals functional compartmentation of the gut in wood-feeding higher termites (Nasutitermes spp.). *Applied and Environmental Microbiology*, 78(13), 4691-4701.
28. Koike, S., and Kobayashi, Y. (2001). Development and use of competitive PCR assays for the rumen

cellulolytic bacteria: Fibrobacter succinogenes, Ruminococcus albus and Ruminococcus flavefaciens. *FEMS Microbiology Letters*, 204(2), 361-366.

29. Kuhad, R. C., Gupta, R., and Singh, A. (2011). Microbial cellulases and their industrial applications. *Enzyme research*, 1-10.
30. Kuhnigk, T., and König, H. (1997). Degradation of dimeric lignin model compounds by aerobic bacteria isolated from the hindgut of xylophagous termites. *Journal of Basic Microbiology*, 37(3), 205-211.
31. Kuhnigk, T., Branke, J., Krekeler, D., Cypionka, H., and König, H. (1996). A feasible role of sulfate-reducing bacteria in the termite gut. *Systematic and Applied Microbiology*, 19(2), 139-149.
32. Leadbetter, J. R., and Breznak, J. A. (1996). Physiological ecology of Methanobrevibacter cuticularis sp. nov. and Methanobrevibacter curvatus sp. nov., isolated from the hindgut of the termite Reticulitermes flavipes. *Applied and Environmental Microbiology*, 62(10), 3620-3631.
33. Leadbetter, J. R., Schmidt, T. M., Graber, J. R., and Breznak, J. A. (1999). Acetogenesis from H2 plus CO2 by spirochetes from termite guts. *Science*, 283(5402), 686-689.
34. Lilburn, T. G., Kim, K. S., Ostrom, N. E., Byzek, K. R., Leadbetter, J. R., and Breznak, J. A. (2001). Nitrogen fixation by symbiotic and free-living spirochetes. *Science*, 292(5526), 2495-2498.
35. Liu, Y., and Whitman, W. B. (2008). Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Annals of the New York Academy of Sciences*, 1125(1), 171-189.
36. Mannesmann, R., and Piechowski, B. (1989). Verteilungsmuster von Gärkammerbakterien einiger Termitenarten. *Mat Org*, 24, 161-177.
37. Morris, J. J., Lenski, R. E., and Zinser, E. R. (2012). The Black Queen Hypothesis: evolution of dependencies through adaptive gene loss. *Amercan Society for Microbiology: MBio*, 3(2), 10-1128.
38. Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., and Ladisch, M. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*, 96(6), 673-686.
39. Nagavallemma, K. P., Wani, S. P., Lacroix, S., Padmaja, V. V., Vineela, C., Rao, M. B. and Sahrawat, K. L. (2006). Vermicomposting: recycling wastes into valuable organic fertilizer. *Journal of SAT Agricultural Research*, 2(1), 1-17.
40. Nakashima, K., Watanabe, H., Saitoh, H., Tokuda, G., and Azuma, J. I. (2002). Dual cellulose-digesting system of the wood-feeding termite, Coptotermes formosanus Shiraki. *Insect Biochemistry and Molecular Biology*, 32(7), 777-784.
41. Ni, J., and Tokuda, G. (2013). Lignocellulose-degrading enzymes from termites and their symbiotic microbiota. *Biotechnology Advances*, 31(6), 838-850.
42. Ohkuma, M. (2003). Termite symbiotic systems: efficient bio-recycling of lignocellulose. *Applied Microbiology and Biotechnology*, 61(1), 1-9.
43. Pandey, A., Biswas, S., Sukumaran, R. K., and Kaushik, N. (2009). Study on availability of Indian

biomass resources for exploitation: a report based on a nation-wise survey. *TIFAC, New Delhi,* 105.

44. Patel, A. K., Dixit, P., Pandey, A., and Singhania, R. R. (2020). Promising enzymes for biomass processing. *Biomass, Biofuels, Biochemicals*, 245-271.
45. Pedroza Matute, S., and Iyavoo, S. (2023). Exploring the gut microbiota: lifestyle choices, disease associations, and personal genomics. *Frontiers in Nutrition*, 10, 1225120.
46. Pope, P.B., Mackenzie, A.K., Gregor, I., Smith, W., Sundset, M.A., McHardy, A.C., Morrison, M. and Eijsink, V.G. (2012). Metagenomics of the Svalbard reindeer rumen microbiome reveals abundance of polysaccharide utilization loci. *Plos One*, 7(6), 38571.
47. Potrikus, C. J., and Breznak, J. A. (1980). Uric acid-degrading bacteria in guts of termites [Reticulitermes flavipes (Kollar)]. *Applied and Environmental Microbiology*, 40(1), 117-124.
48. Prillinger, H., Messner, R., König, H., Bauer, R., Lopandic, K., Molnar, O., Dangel, P., Weigang, F., Kirisits, T., Nakase, T. and Sigler, L. (1996). Yeasts associated with termites: a phenotypic and genotypic characterization and use of coevolution for dating evolutionary radiations in asco-and basidiomycetes. *Systematic and Applied Microbiology*, 19(2), 265-283.
49. Saha, B. C. (2003). Hemicellulose bioconversion. *Journal of Industrial Microbiology and Biotechnology*, 30(5), 279-291.
50. Sánchez, C. (2009). Lignocellulosic residues: biodegradation and bioconversion by fungi. *Biotechnology Advances*, 27(2), 185-194.
51. Schäfer, A., Konrad, R., Kuhnigk, T., Kämpfer, P., Hertel, H., and König, H. (1996). Hemicellulose-degrading bacteria and yeasts from the termite gut. *Journal of Applied Microbiology*, 80(5), 471-478.
52. Schopf, J. W. (2006). Fossil evidence of Archaean life. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 361(1470), 869-885.
53. Schultz, J. E., and Breznak, J. A. (1978). Heterotrophic bacteria present in hindguts of wood-eating termites [Reticulitermes flavipes (Kollar)]. *Applied and Environmental Microbiology*, 35(5), 930-936.
54. Singhania, R. R., Patel, A. K., Sukumaran, R. K., Larroche, C., and Pandey, A. (2013). Role and significance of beta-glucosidases in the hydrolysis of cellulose for bioethanol production. *Bioresource Technology*, 127, 500-507.
55. Singhania, R. R., Sukumaran, R. K., Patel, A. K., Larroche, C., and Pandey, A. (2010). Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases. *Enzyme and Microbial Technology*, 46(7), 541-549.
56. Suen, G., Weimer, P.J., Stevenson, D.M., Aylward, F.O., Boyum, J., Deneke, J., Drinkwater, C., Ivanova, N.N., Mikhailova, N., Chertkov, O. and Goodwin, L.A. (2011). The complete genome sequence of Fibrobacter succinogenes S85 reveals a cellulolytic and metabolic specialist. *Plos One*, 6(4), 18814.
57. Sukumaran, R. K., Singhania, R. R., and Pandey, A. (2005). Microbial cellulases-production, applications and challenges. *Journal of Scientific and Industrial Research*, 64(11), 832-844.

58. Sun, Y., and Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology*, 83(1), 1-11.
59. Szewzyk, U., Szewzyk, R., and Stenström, T. A. (1994). Thermophilic, anaerobic bacteria isolated from a deep borehole in granite in Sweden. *Proceedings of the National Academy of Sciences*, 91(5), 1810-1813.
60. Thauer, R. K., Kaster, A. K., Seedorf, H., Buckel, W., and Hedderich, R. (2008). Methanogenic archaea: ecologically relevant differences in energy conservation. *Nature Reviews Microbiology*, 6(8), 579-591.
61. Thomas, F., Hehemann, J. H., Rebuffet, E., Czjzek, M., and Michel, G. (2011). Environmental and gut bacteroidetes: the food connection. *Frontiers in Microbiology*, 2, 93.
62. Tokuda, G., Watanabe, H., Hojo, M., Fujita, A., Makiya, H., Miyagi, M., Arakawa, G. and Arioka, M. (2012). Cellulolytic environment in the midgut of the wood-feeding higher termite Nasutitermes takasagoensis. *Journal of Insect Physiology*, 58(1), 147-154.
63. Upadhyaya, S. K., Manandhar, A., Mainali, H., Pokhrel, A. R., Rijal, A., Pradhan, B., and Koirala, B. (2012). Isolation and characterization of cellulolytic bacteria from gut of termite. *Rentech Symposium Compendium*, 1(4), 14-18.
64. Wan, C., and Li, Y. (2012). Fungal pretreatment of lignocellulosic biomass. *Biotechnology Advances*, 30(6), 1447-1457.
65. Warnecke, F., Luginbühl, P., Ivanova, N., Ghassemian, M., Richardson, T.H., Stege, J.T., Cayouette, M., McHardy, A.C., Djordjevic, G., Aboushadi, N. and Sorek, R. (2007). Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature*, 450(7169), 560-565.
66. Weimer, P. J. (1998). Manipulating ruminal fermentation: a microbial ecological perspective. *Journal of Animal Science*, 76(12), 3114-3122.
67. Wenzel, M., Schönig, I., Berchtold, M., Kämpfer, P., and König, H. (2002). Aerobic and facultatively anaerobic cellulolytic bacteria from the gut of the termite Zootermopsis angusticollis. *Journal of Applied Microbiology*, 92(1), 32-40.
68. Xiao, Z., Storms, R., and Tsang, A. (2004). Microplate-based filter paper assay to measure total cellulase activity. *Biotechnology and Bioengineering*, 88(7), 832-837.
69. Zhao, F., Yi, Y., and Lü, X. (2021). Essential process and key barriers for converting plant biomass into biofuels. *Advances in 2nd Generation of Bioethanol Production*, 53-70.
70. Zhao, X., Zhang, L., and Liu, D. (2012). Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. *Biofuels, Bioproducts and Biorefining*, 6(4), 465-482.
71. Zhu, J. Y., Pan, X. J., Wang, G. S., and Gleisner, R. (2009). Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. *Bioresource Technology*, 100(8), 2411-2418.