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Termite Digestomes as a Potential Source of Symbiotic Microbiota for Lignocelluloses Degradation: A Review

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Abstract: Termites thrive in great abundance in terrestrial ecosystems and the symbiotic gut microbiota play important roles in digestion of lignocelluloses and nitrogen metabolism. Termites are excellent models of biocatalysts as they inhabit dense microbes in their guts that produce digestive enzymes to decompose lignocelluloses and convert it to end products such as sugars, hydrogen, and acetate. Different of digestive system between lower and higher termites which lower termites dependent on their dual decomposing system, consisting of termite's own cellulases and gut's protists. Higher termites decompose cellulose using their own enzymes, because of the absence of symbiotic priotists. Termite gut prokaryotes efficiently support lignocelluloses degradation. In this review, a brief overview of recent experimental works, development and commercialization is discussed. Significant progress has been made to isolate cellulolytic strains from termites and optimise the digestion efficiency of cellulose. Future perspective should emphasize the isolation of cellulolytic strains from termites, genetically modifying or immobilization of the microbes which produce the desired enzyme and thus benefits on the microbiology and biotechnology.

Key words: Termite, lignocelluloses, polysaccharides, microbes, bacteria, flagellate

INTRODUCTION

Termites are one of the most successful wood-degraders on Earth, tunnelling and chewing on wood for millions of years (Radek, 1999). These insects made their first imprint as early as the Cretaceous period with the unearthing of oldest termite fossils of approximately 100 million years old. Termites are representing one of the social insects. They are grouped in ancient insect order Isoptera and they are closely related to cockroach and mantises (Radek, 1999; Kricher, 2011). Throughout the world, termites are traceable along the equators, in the tropical forests and Mediterranean shrublands, where dense biomass and the greatest biodiversity take place (Abensperg-Traun and Milewski, 1995). As mankind are fearful for their structures and crops damaging capabilities, the nature graciously welcomes these wood-grazers. Termites also account for severe damage to wood in use. In order to enhance the resistance ability of wood towards termite's invasion, application of termite repellent and post heat treatment on wood are now widely used (H'ng *et al*., 2012). Nevertheless, ecologically speaking, termites are beneficial insects that play an essential role in recycling nutrients, forming habitats, aerating and improving soils, and as food for countless predators (Radek, 1999; Kricher, 2011).

Termites survive on any wood and lignocellulose materials (H'ng and Chin, 2008; Lee *et al*., 2013). They coverts the cellulose of wood and lignocellulosic materials into carbohydrates before translated into energy. Cellulose is part of plant structures and the basic structural components of cell wall. Cellulose is a polysaccharide, a tough linear chain of glucose joined by β-1,4-glycosidic and hydrogen bonds. To disintegrate this polysaccharide into simple glucose, termites are loaded with as much as 250 different species of microorganisms in their relatively tiny guts (Nadin, 2007). Nonetheless, not all the microorganisms in the gut of termites function in cellulosic degradation. Each of the microorganisms is responsible in breaking down specific components of the plant structures to different end products. The termites provide the needed settlement for the microbes and feed on wood, while the microbes digest the food for their hosts in return. Such exchange reflects a mutual symbiotic relationship that benefits both the host and symbiont.

The microbial organisms inside the termite's gut, however, do not develop by themselves. Before termites

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could start munching on wood, they need to engage in trophallaxis (Wilson, 1971; Machida *et al*., 2001). In other words, they are exchange food and digestive fluid through mouth-to-mouth (stomodeal) or feeding on each other's faeces (proctodeal) (Quarcoo, 2009). Such fluid contains the essential nutrients and endosymbionts that are passed on to the younger instars by the mature termites (James, 2008). Termites are lost most of their symbionts at every molt, and termites have to feed themselves with the recycled symbionts via proctodeal trophallaxis (James, 2008). They rely on symbiotic bacteria embedded on their surfaces to produce some of the digestive enzymes to degrade cellulose for their termite host (Radek, 1999). The resulting glucose and acetate are then absorbed by the termites as a primary source of energy (Radek, 1999; Ohkuma, 2003)

Termites' successful survival on cellulose-rich diet suggests that significant decomposition of wood components is taking place in the gut. Almost 90% of the cellulose in wood is turned into acetate (Nadin, 2007). Many scientists are now convinced that termite's gut resembles an efficient living bioreactor. Different species of the microbes in termite gut have different needs and release different end-products but they share a common goal-to degrade lignocelluloses into different applicable products. By turning the energy-rich cellulose into acetate and glucose, the symbiotic microbial community in termite's gut could be the key to generate customised enzymatic-cocktails to apply in the biomass processing industry. Termites are therefore a potentially powerful catalyst for feasible bioethanol production.

DISTRIBUTION OF TERMITES

According to the theoretical and empirical data from Models of Population Dynamics and Models of Population Oscillations, the maximum of termite population would be gotten during 2020s years and world population may be until 10^7-10^8 billion (Sapunov, 2008). In the high population of termites, there are 2,650 species of termites, and the majority of them occur only within tropical and sub-tropical latitudes (Kricher, 2011). Termites are playing significant roles in food webs and influence the provision of ecosystem such as decomposition. They occur in vast numbers in tropical regions, which exceed 100 g m^{-2} and 10,000 individuals m^{-2} in the tropical forests (Eggleton *et al*., 1996). But their area prolongs to increase included Italy, New Zealand, and Australia.

In addition, the distribution of Drywood termites, Subterranean termites, Formosan termites, and Dampwood termites is varying by region. Drywood termites are live in the countries that do not reach freezing temperatures during winter and they are found along East Coast from the Mid-Atlantic States to South Florida, along the Gulf Coast, through the Southwest into California, and in Hawaii. The Subterranean termites which live in the soil underground; are able to survive in wide range of temperatures. In US, the subterranean termites are found in every state, except Alaska. As a pest of forest tree, Dampwood termites are rarely damage wood in buildings. They do not nest in the soil but mainly nest in decaying stumps, logs and eucalypt trees.

In Malaysia alone, it is estimated there could be 180 species of termites representing 48 genera that live in different habitats in the country (Tho, 1992). A termite can correspond to up biomass of invertebrates in decomposing trunks (Bandeira and Torres, 1985). At least ten identified species are known to invade wooden structures, paper products, cotton clothings or ornamental trees. *Coptotermes gestroi* (Asian subterranean termite) is the most common and aggressive wood-feeding termite species found in Malaysia and was reported to cause major damages (60-70%) specifically to interior wooden structures, followed by *C. curvignathus* contributing to about 20% of the total structural damage and attacking living trees as well as rubber, oil palm and coconut plantations (Yeoh and Lee, 2007). Other essentially threatening species in Malaysia include *Odontotermes* sp., *Schedorhinotermes* sp., *Macrotermes gilvus*, *Nasutitermes* sp., *Microcerotermes crassus*, *Globitermes sulphurous*, *Macrotermes carbonarius* and *Microtermes* spp.

LOWER AND HIGHER TERMITES

The order Isoptera of termites is phylogenetically classified into seven families and fifteen subfamilies (Lee and Wood, 1971). The families are: (1) Mastotermitidae, (2) Kalotermitidae, (3) Termopsidae, (4) Hodotermitidae, (5) Rhinotermitidae, (6) Serritermitidae, and (7) Termitidae. The Mastotermitidae, Kalotermitidae, Termopsidae, Hodotermitidae and Rhinotermitidae families are identified as the lower termites, whilst the Serritermitidae, and Termitidae families are acknowledged as the higher termites.

The taxonomy of lower and higher termites is based on the termites' stage of evolution, in terms of their behaviour and anatomically. The main difference between higher and lower termites is the gut of lower termites comprises with protozoa, while the gut of higher termites is lack of protozoa (Varma *et al*., 1994). In the digestive tracts of lower termites, degrading of cellulose is depend on flagellates, yeasts and bacteria (Breznak and Brune, 1994; Varma *et al*., 1994; Konig *et al*., 2002) including by the termite's own cellulases (Tokuda *et al*., 1999; Watanabe *et al*., 1998; Tokuda *et al*., 2002). The higher termites are able to decompose cellulose by using their own enzymes (Ohkuma, 2003) through the gut passage.

Scientists discovered that diets and digestion of cellulose seems to differ between higher and lower termites. In addition, most of the species of lower termites are wood-feeding termite. The digestion resistance of woods causes the termites to favour wood that has been attacked by fungi. With the presence of fungi mycelia, the woods are richer in protein content and easier to be utilised by termites. Lower termites, such as *Coptotermes lacteus* and *Reticulitermes speratus*, are long known to utilise gut protozoa for cellulose digestion in addition to synthesising its own cellulases (O'Brien *et al*., 1979; Kudo *et al*., 1998). By digestion of lignocelluloses and extract their dietary requirements from food resources, it create the symbiotic relationship of termites with the intestinal flagellates and bacteria contained in a large dilatation of their hindgut, which is the paunch.

By contrast, higher termites do not harbour flagellates and typically lack protists hence show different feeding habits. Higher termites decompose cellulose efficiently in the absence of hindgut flagellate protozoa (Li *et al*., 2006) which are recognized sources of cellulase and hemicellulases in lower termites (Warnecke *et al*., 2007). The Termitidae ingest a wide range of materials include leaves, roots, grass, dung, and soil (humus) (Wood and Johnson, 1986). In addition, there are two groups in Termitidae, fungus-cultivating species and non-fungus-cultivating species. The fungus-cultivating species of termites are able to build a large fungal garden in their nests. The garden is constructed by assembly partially digested plant materials and further digested by fungal mycelium (Wood and Thomas, 1989). Hence, the termite workers eat the fungus comb which contained nutrition. In addition to the direct nutritional value, the ingested fungi may deliver "missing enzyme' essential for the completion of cellulose digestion (Martin, 1987, 1991, 1992).

TERMITE AND ITS DEGRADATION ON LIGNOCELLULOSIC MATERIAL

Lignocellulose can serve as a biomass material for a number of industrial biorefinery process, namely pyrolysis, hydrolysis, gasification to value-added products such as glucose, xylose, starch, ethanol (Kim and Dale, 2004; Scharf and Tartar, 2008; Chin *et al*., 2010, 2011; H'ng *et al*., 2011; Tay *et al*., 2013; Chin and H'ng, 2013). The main challenge facing lignocellulosic materials utilization is the energy, costs input involved in treatment and production processes. Therefore, researches have expanded on the potential of the termite-based biological pretreatment strategy for use in lignocelluloses degradation.

Termites efficiently digest lignocellulose using their endogenous and digestive enzymes in the termite gut (Breznak and Brune, 1994; Watanabe *et al*., 1998; Ohkuma 2003; Scharf and Tartar, 2008; Tartar *et al*., 2009). The symbiotic digestion of polysaccharides by termites is involving a complex of host and its gut microbiota, which comprises bacteria, fungi, protozoa to degrade cellulose and hemicelluloses (Radek, 1999; Brune, 2009). The microbial community in the gut of termites has been attracting many scientists due to their symbiotic digestion mechanisms in the hindgut are largely controlled by the symbionts (Brune, 2009). According to previous reports suggested that termites could efficiently decompose lignocelluloses within a day by degrading 74-99% of the cellulose, 65-87% of the hemicellulose as well as 5-83% of the lignin which are able to removes most neutral polysaccharides and more than half of the acidic sugars (Breznak and Brune, 1994; Konig *et al*., 2006; Sun, 2008). Nevertheless, cows decompose only 30-40% of polysaccharides in their forage (Brune and Ohkuma, 2011).

Sound wood is most difficult to digest because the polysaccharides of the secondary plant cell wall are embedded in an amorphous resin of phenolic polymers which causing the barrier to enzymatic attack of the polysaccharides (Brune, 2009). Therefore, an efficient of symbiont-derived digestive enzyme in cellulolytic system is required to the polysaccharides degradation (Scharf and Boucias, 2010). Therefore, the incredible metabolic capability of the termite gut is potential biocatalyst in aerobic fermentative degradation of carbohydrates, and in metabolism of lignin-derived aromatic compounds (Brune, 1998).

ROLE OF TERMITE GUT MICROBIOATA

Let us take a look at how is the role of termite gut microbiota in lignocelluloses digestion and may bring potentially beneficial in industrials application. For instance, the lignocelluloses digestion is highly achieved in the termite gut as the termite's digestome is apparent as a pool of host and symbiont genes (Scharf and Tartar, 2008; Tartar *et al*., 2009).

The cellulose activity in the hindgut of termites is attributed to cellulose-degradation bacteria. (Schwarz, 2001). Termite gut contains a lot of microbes which can digest cellulose such as the Gram-positive bacteria:

Bacillus, *Paenibaccillus*, *Streptomyces*, *Actinobacteria* group and Gram-negative bacteria: *Pseudomonas, Acinetobacter*; *Facultative* microbe: *Serratia marcescens*, *Enterobacter aerogens*, *citrobacter farmer*. Gram-positive strains related to *Cellulomonas*, *Bacillus* and *Paenibacillus* showed highest CMC-degrading potential. Wenzel *et al*. (2002) argued that the cellulolytic bacteria are taking over the role of flagellates in higher termites.

Most of the gut bacteria are necessary for the survival of their hosts even though they are indirectly involved in cellulose degradation in termites gut (Slaytor, 1992; Radek, 1999). In a termite's gut, cellulose is broken down into simple sugar by certain cellulolytic species, subsequently metabolised to form pyruvate. Other microbial species collaborate in turn to transform the pyruvate to different end-products, such as CO_2, acetate, methane or ethanol, depending on availability of oxygen supply (Nadin, 2007). While concentrations in the midgut are aerobic, oxygen concentrations are low in the hindgut (Radek, 1999). Eventually the transformation cycle repeats again on another type of substrates. As much as 250 microbial species are adapted to live in a termite's gut together, but each is individually involved in different transformation of varying substrates.

Termites are mostly feed on the dead grass, wood, and other plant material to obtain essential energy from the digestion of cellulose (Andersen and Jacklyn, 1993; Pearce, 1997). Therefore, it is an opportunity of termite biomass used as food sources for the aquaculture, pig, and poultry industries. At present, termite microbes have been proven useful in poultry feed additive. Purwadaria *et al*. (2003) detected cellulolytic activities in the fresh extract of termites (*Glyptotermes montanus*) that increases the digestion of poultry feedstuffs containing high lignocelluloses such as rice bran, wheat pollard, Palm Oil Mill Effluent (POME), Palm Kernel Cake (PKC), corn and soybean meals. Rich of protein in termite gut replace 50% fishmeal in formulations and it is a useful supplement for family poultry. Nutrients left behind in termite wastes may also be useful for horticultural purposes, particularly compost which potential be a novel resource for organic biofertilizers (Chai *et al*., 2013; Peng *et al*., 2013).

Current studies showed that termite symbionts have involved as cellulolytic or lignin-derived component and degradation of aromatic hydrocarbons compounds. Hence, that would be useful for industrial application such as biomass consumption, environmental remediation and fine-chemicals production. Advances in the conversion technology will add value to existing biochemicals production and boost exciting economic opportunities of bio-based applications as well as fuels, chemicals and pharmaceuticals. Despite slower reaction time and careful control of microbial growth conditions, biological system involving termite symbionts appears to be more appealing (Sun and Cheng, 2002; Zheng *et al*., 2009) for lignocelluloses degradation.

CURRENT RESEARCH AND FUTURE PERSPECTIVE ON TERMITE'S GUT MICROBIOATA

As discussed, termite lignocellulose digestion has been considered as a gut-symbiont-mediated process. The termite gut is explored as a source of novel microorganisms and may bring many benefits to large scale industrial applications (Tokuda *et al*., 2004). In fact, the symbiotic association of termites with their diversity intestinal macrobiotic is receiving interests from various aspects such as microbiology, biochemistry, protozoology, insect physiology and ecology, socio-biology, evolutionary biology, and even in atmospheric chemistry (Sanderson, 1996; Higashi and Abe, 1997; Sugimoto *et al*., 2000). Hence, researches have been further expanded on the anaerobic food web and nitrogen metabolism in the termite gut.

In addition, in microbial gut of termite, also include nitrogen fixing bacteria (Benemann, 1973; Breznak *et al*., 1973; French *et al*., 1976; Potrikus and Breznak, 1977; Prestwich and Bentley, 1981). Nitrogen fixation by termite gut microbes has been known for years ago (Breznak, 2000) and nitrogen fixation contributes as much as 60% of N in some termite colonies (Tayasu *et al*., 1994). Since the nitrogen compound are insufficient in wood and soil, the nitrogen fixing bacteria (e.g., Enterobactor, Rhizobium, Desulfovibrio) is play a vital role in symbiotic community (Lovelock *et al*., 1985; Radek, 1999). However, the wood-feeding termites are strongly nitrogen limited (Brune and Ohkuma, 2011). Researchers showed that the hindgut microbiota of termites includes a morphologically diverse population of N-fixing Spirochetes bacteria which have reached 50% of all prokaryotes (Paster *et al*.,1996; Breznak, 2002). The spirochetes are involved in acetogenesis and N_2 fixation process to provide the carbon, nitrogen and energy needs of their termite host. The N-fixing bacteria produce amino acids that are partly liberated and may be used by termites and flagellates. Nonetheless, the metabolic role of Spirochetes is entirely unknown (Radek, 1999). Hence, more researches are needed to study the metabolic properties of Spirochetes especially the spirochetes contribution to H_2/CO_2-acetogenesis and N_2 fixation. Next, the study on the properties of spirochetes (or of the termite gut itself) enables them to become such a prominent component of the microbiota is needed in the field of researches also.

In addition, there are fermenting bacteria also found in the termite gut from the anaerobic food web. The low concentrations of soluble sugars and the accumulation of their metabolites in the hindgut fluid of termites indicate that polysaccharides depolymerization is coupled to the fermentative degradation of its hydrolysis products (Brune and Ohkuma, 2011). The fermenting bacteria (e.g., of the genera Streptococcus, Bacteroides, Fusobacterium, and Lactobacillus), profit by the low amount of mono-, di-, and oligosaccharides, liberated by the flagellates (Breznak, 1984; Radek, 1999). The fermentation of cellulose is following the equation as shown as below (Odelson and Breznak, 1985; Brune and Ohkuma, 2011):

$$[C_6H_{12}O_6] + 2H_2O \rightarrow 2CH_3COO^- + 2H^+ + 2CO_2 + 4H_2$$

The metabolic end product of the anaerobic fermentation food web is acetate (termite's fuel), and other organic acid, which can be transported across the gut wall for reabsorbed by the host and form the basis for its energy metabolism (Radek, 1999; Brune and Ohkuma, 2011). According to the few researches, two species of fermenting bacteria were identified which include *Acetonema longum* by Kane and Breznak (1991) and *Enterococcus* sp. by Tholen *et al.* (1997).

In the new study, researchers have suggested the termite's enzyme could be boon to cellulosic ethanol by fermentation process. Researcher claim that a type of bacteria that helps termite digests wood could be a key to making ethanol economically from non-food crops such as wood and grass (REF). Meanwhile, wood decay in the guts of termites generates hydrogen gas from lignin as a key intermediate product. This explosive, energy-rich hydrogen gas can be combined with ethyl acetate to make ethanol or provide energy for gasification. As a result, research is currently applied to understanding the interactions of lignocelluloses degradation and symbiotic microbes in termites gut to provide innovations in technology to address this challenge for producing ethanol (Nadin, 2007; Brune, 1998; Scharf and Boucias, 2010; Li *et al.*, 2012). Bioethanol production with emphasis on cellulosic ethanol brings a scientific challenge of achieving cost effective degradation of complex cellulosic biomass (pre-treatment and hydrolysis stages) (McMillan, 1994). Thus, the microbes living in termites gut provide a fast and efficient hydrolysis of biomass if harnessed and applied appropriately to produce cellulosic ethanol at an industrial scale.

For increased efficiency and reduced production costs, future findings should highlight the isolation of cellulolytic strains or microbial species from termites, genetically modifiying or immobilization of microbes which produce the desired enzymes. As such, termite gut digestomics is a relatively new area of research. In the future, termite guts can potentially advance the bioconversion of lignocellulosic materials to valuable product such as fuel as it is an effective, economic, and sustainable ways.

CONCLUSION

Termites are regarded as harmful insects because of their ability in destroying various materials including lignocellulosis biomass. The termites' digestion process on the cellulose is fast and efficient which typically achieving 95% conversion in 24 h or less. However, the microbiology mechanism is different between the two classes of termites which are 'lower termites' and 'higher termites'. In which, the differences of microbiology of lower termites and higher termites may also differ in their role in degrading cellulose. In recent years, termites have captured the interests of researchers from various disciplines to investigate their gut microbial symbionts and their incredible ecological importance to the global carbon cycle. The ability of termites to hydrolyse a broad assortment of chemical bonds and break down the lignocelluloses into monomer sugars quickly has astonished researchers. Apparently, the development of low-cost enzymatic approach with termites is promising and ecological to accomplish the bioconversion of lignocelluloses into useful products such as glucose and ethanol.

REFERENCES

Abensperg-Traun, M. and A.V. Milewski, 1995. Abundance and diversity of termites (Isoptera) in imburnt versus burnt vegetation at the Barrens in Mediterranean Western Australia. Aust. J. Ecol., 20: 413-417.

Andersen, A. and P. Jacklyn, 1993. Termites of the Top End. CSIRO Publishing, Darwin, Australia, ISBN-13: 9780643102835, Pages: 31.

Bandeira, A.G. and M.F.P. Torres, 1985. Abundancia e distribuicao de invertebrados do solo em ecossitemas da Amazonia Oriental. O papel ecologico dos cupins [Abundance and distribution of soil invertebrates in ecosystems of Eastern Amazonia. The ecological role of termites]. Boletim do Museu Paraense Emilio Goeldi Serie Zoologica, 2: 13-38.

Benemann, J.R., 1973. Nitrogen fixation in termites. Science, 181: 164-165.

Breznak, J.A., W.J. Brill, J.W. Mertins and H.C. Coppel, 1973. Nitrogen fixation in termites. Nature, 244: 577-580.

Breznak, J.A., 1984. Biochemical Aspects of Symbiosis Between Termites and their Intestinal Microbiodata. In: Invertebrate-Microbial Interactions, Anderson, J.M., A.D.M, Rayner and D.W.H. Watts (Eds.). Cambridge University Press, UK., pp: 173-204.

Breznak, J.A. and A. Brune, 1994. Role of microorgamisms in the digestion of lignocellulose by termites. Annu. Rev. Entomol., 39: 453-487.

Breznak, J.A., 2000. Ecology of Prokaryotic Microbes in the Guts of Wood and Litter Feeding Termites. In: Termites: Evolution, Sociality, Symbioses, Ecology. Abe, T., D.E. Bignell and M. Higashi (Eds.). Springer, New York, USA., ISBN: 9780792363613, pp: 209-231.

Breznak, J.A., 2002. Phylogenetic diversity and physiology of termite gut spirochetes. Integr. Comp. Biol., 42: 313-318.

Brune, A. and M. Ohkuma, 2011. Role of the Termite Gut Microbiota in Symbiotic Digestion. In: Biology of Termites: A Modern Synthesis, Bignell, D.E., Y. Roisin and N. Lo (Eds.). Springer, Dordrecht, The Netherlands, ISBN-13: 9789048139774, pp: 439-477.

Brune, A., 1998. Termite guts: The world's smallest bioreactors. Trends Biotechnol., 16: 16-21.

Brune, A., 2009. Symbionts Aiding Digestion. In: Encyclopedia of Insects, Resh, V.H. and R.T. Carde (Eds.). 2nd Edn., Academic Press, New York, USA., ISBN: 9780080920900, pp: 978-983.

Chai, E.W., P.S. H'ng, S.H. Peng, W.M. Wan-Azha, K.L. Chin, M.J. Chow and W.Z. Wong, 2013. Compost feedstock characteristics and ratio modelling for organic waste materials co-composting in Malaysia. Environ. Technol., 34: 2859-2866.

Chin, K.L., P.S. H'ng, L.J. Wong, B.T. Tey and M.T. Paridah, 2010. Optimization study of ethanolic fermentation from oil palm trunk, rubberwood and mixed hardwood hydrolysates using *Saccharomyces cerevisiae*. Bioresour. Technol., 101: 3287-3291.

Chin, K.L., P.S. H'ng, L.J. Wong, B.T. Tey and M.T. Paridah, 2011. Production of glucose from oil palm trunk and sawdust of rubberwood and mixed hardwood. Applied Energy, 88: 4222-4228.

Chin, K.L. and P.S. H'ng, 2013. A Real Story of Bioethanol from Biomass: Malaysia Perspective. In: Biomass Now-Sustainable Growth and Use, Matovic, M.D. (Ed.). InTech Publisher, Rijeka, Croatia, pp: 329-346.

Eggleton, P., D.E. Bignell, W.A. Sands, N.A. Mawdsley, J.H. Lawton, T.G. Wood and N.C. Bignell, 1996. The diversity, abundance and biomass of termites under differing levels of disturbance in the Mbalmayo forest reserve, Southern Cameroon. Phil. Trans. Royal Soc. London B, 351: 51-68.

French, J.R., G.L. Tuner and J.F. Bradbury, 1976. Nitrogen fixation by bacteria from the hindgut of termites. J. Gen. Microbiol., 95: 202-206.

H'ng, P.S. and K.L. Chin, 2008. Solid hardwood flooring resistance to termites (*Coptertermes curvignathus*) under laboratory condition. Malaysian For., 71: 131-137.

H'ng, P.S., L.J. Wong, K.L. Chin, E.S. Tor, S.E. Tan, B.T. Tey and M. Maminski, 2011. Oil palm (*Elaeis guineensis*) trunk as a resource of starch and other sugars. J. Applied Sci., 11: 3053-3057.

H'ng, P.S., S.H. Lee and W.C. Lum, 2012. Effect of post heat treatment on dimensional stability of UF bonded particleboard. Asian J. Applied Sci., 5: 299-306.

Higashi, M. and T. Abe, 1997. Global Diversification of Termites Driven by the Evolution of Symbiosis and Sociality. In: Biodiversity: An Ecological Perspective, Abe, T., S.A. Levin and M. Higashi (Eds.). Vol. 12, Springer, New York, USA., pp: 83-112.

James, L.N., 2008. Alimentary Canal and Digestion. In: Encyclopedia of Entomology, John, L.C. (Ed.). Vol. 4, Springer, New York, USA., ISBN-13: 9781402062421, pp: 111-118.

Kane, M.D. and J.A. Breznak, 1991. *Acetonema Longum* gen.nov.sp.nov. an H_2/CO_2 acetogenic bacterium from the termite, *Pterotermes occidentis*. Arch. Microbiol., 156: 91-98.

Kim, S. and B.E. Dale, 2004. Global potential bioethanol production from wasted crops and crop residues. Biomass Bioenergy, 26: 361-375.

Konig, H., J. Frohlich, M. Berchtold and M. Wenzel, 2002. Diversity and microhabitats of the hindgut flora of termites. Rec. Res. Microbiol., 6: 125-156.

Konig, H., J. Frochlich and H. Hertel, 2006. Diversity and Lignocellulolytic Activities of Cultured Microorgamisms. In: Intestinal Microorganisms of Termites and Other Inverterbrates, Konig, H. and A. Verma (Eds.). Springer-Verlag, Berlin, Germany, pp: 271-301.

Kricher, J.C., 2011. Tropical Ecology. Princeton University Press, UK., ISBN-13: 9781400838950, pp: 359-390.

Kudo, T., M. Ohkuma, S. Moriya, S. Noda and K. Ohtoko, 1998. Molecular phylogenetic identification of the intestinal anaerobic microbial community in the hindgut of the termite, Reticulitermes speratus, without cultivation. Extremophiles, 2: 155-161.

Lee, K.E. and T.G. Wood, 1971. Termites and Soils. Academic Press, New York, USA., Pages: 251.

Lee, S.H., P.S. H'ng, T.L. Peng and W.C. Lum, 2013. Response of *Coptotermes curvignathus* (Isoptera: Rhinotermitidae) to formaldehyde catcher-treated particleboard. Pak. J. Biol. Sci., 16: 1415-1418.

Li, L., J. Frohlich and H. Konig, 2006. Cellulose Digestion in the Termite Gut. In: Intestinal Microorganisms of Termites and Other Inverterbrates, Konig, H. and A. Verma (Eds.), Springer-Verlag, Berlin, Germany, pp: 221-241.

Li, H.J., J. Lu and J. Mo, 2012. Physiochemical lignocellulose modification by the formasan subterranean termite *Coptotermes formosanus shiraki* (Isoptera: Rhinotemitidae) and its potential uses in the production of biofuels. BioResources, 7: 675-685.

Lovelock, M., R.W. O'Brien and M. Slaytor, 1985. Effect of laboratory containment on the nitrogen metabolism of termites. Insect Biochem., 15: 503-509.

Machida, M., O. Kitade, T. Miura and T. Matsumoto, 2001. Nitrogen recycling through proctodeal trophallaxis in the Japanese damp-wood termite *Hodotermopsis japonica* (Isoptera, Termopsidae). Insectes Sociaux, 48: 52-56.

Martin, M.M., 1987. Invertebrate-Microbial Interactions: Ingested Fungal Enzymes in Arthropod Biology. Comstock, Ithaca, New York, USA., ISBN: 13-9780801420559 Pages: 148.

Martin, M.M., 1991. The evolution of cellulose digestion in insects. Phil. Trans. R. Soc. Lond., 333: 281-288.

Martin, M.M., 1992. The Evolution of Insect-fungus associations: From Contact to Stable Symbiosis. Amer. Zool., 32: 593-605.

McMillan, J.D., 1994. Pretreatment of Lignocellulosic Biomass. In: Enzymatic Conversion of Biomass for Fuels Production, Himmel, M.E., J.O. Baker and R.P. Overend (Eds.). American Chemical Society, Washington, DC., USA., ISBN13: 9780841229563, pp: 292-324.

Nadin, E., 2007. For the love of termites. Eng. Sci., 2: 24-31.

O'Brien, G.W., P.C. Veivers, S.E. McEwen, M. Slaytor and R.W. O'Brien, 1979. The origin and distribution of cellulase in the termites, *Nasutitermes exitiosus* and *Coptotermes lacteus*. Insect Biochem., 9: 619-625.

Odelson, D.A. and J.A. Breznak, 1985. Nutrition and growth characteristics of *Trichomitopsis termopsidis*, a cellulolytic protozoan from termites. Applied Environ. Microbiol., 49: 614-621.

Ohkuma, M., 2003. Termite symbiotic systems: Efficient bio-recycling of lignocellulose. Applied Microb. Biotechnol., 61: 1-9.

Paster, B.J., F.E. Dewhirst, S.M. Cooke, V. Fussing, L.K. Poulsen and J.A. Breznak, 1996. Phylogeny of not-yet-cultured spirochetes from termite guts. Applied Environ. Microbiol., 62: 347-352.

Pearce, M.J., 1997. Termites: Biology and Pest Management. CAB International, Wallingford, USA., ISBN-13: 9780851991306, Pages: 172.

Peng, S.H., W.M. Wan-Azha, W.Z. Wong, W.Z. Go, E.W. Chai, K.L. Chin and P.S. H'ng, 2013. Effect of using agro-fertilizers and N-fixing azotobacter enhanced biofertilizers on the growth and yield of corn. J. Applied Sci., 13: 508-512.

Potrikus, C.J. and J.A. Breznak, 1977. Nitrogen-fixing *Enterobacter agglomerans* isolated from guts of wood-eating termites. Applied Environ. Microbiol., 33: 392-399.

Prestwich, G.D. and B.L. Bentley, 1981. Nitrogen fixation in intact colonies of the termite *Nasutitermes corniger*. Oecologia, 49: 249-251.

Purwadaria, T., P.P. Ketaren, A.P. Sinurat and I. Sutikno, 2003. Identification and evaluation of fiber hydrolytic enzymes in the extract of termites (*Glyptotermes montanus*) for poultry feed application. Indo. J. Agric. Sci., 4: 40-47.

Quarcoo, F.Y., 2009. Behavioral toxicology of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae). ProQuest Dissertations and Theses. http://udini.proquest.com/view/behavioral-toxicology-of-the-pqid:1918723221/.

Radek, R., 1999. Flagellates, bacteria and fungi associated with termites: Diversity and function in nutrition: A review. Ecotropica, 5: 183-196.

Sanderson, M.G., 1996. Biomass of termites and their emissions of methane and carbon dioxide: A global database. Global Biogeochem. Cycles, 10: 543-557.

Sapunov, V.B., 2008. Global Dynamics of Termite Population: Modeling, Control and Role in Green House Effect. In: Proceedings of the 6th International Conference on Urban Pests, July 13-16, 2008, Robinson, W.H. and D. Bajomi (Eds). OOK Press, Budapest, Hungary, pp: 389-393.

Scharf, M.E. and A. Tartar, 2008. Termite digestomes as sources for novel lignocellulases. Biofuels Bioprod. Biorefining, 2: 540-552.

Scharf, M.E. and D.G. Boucias, 2010. Potential of termite-based biomass pre-treatment strategies for use in bioethanol production. Insect Sci., 17: 166-174.

Schwarz, W.H., 2001. The cellulosome and cellulose degradation by anaerobic bacteria. Applied Microbiol. Biotechnol., 56: 634-649.

Slaytor, M., 1992. Cellulose digestion in termites and cockroaches: What role do symbionts play? Comp. Biochem. Physioogyl. B Comp. Biochem., 103: 775-784.

Sugimoto, A., D. Bignell and J.A. Macdonald, 2000. Global Impact of Termites on the Carbon Cycle and Atmospheric Trace Gases. In: Termites: Evolution, Sociality, Symbioses, Ecology, Abe, T., D.E. Bignell and M. Higashi (Eds.). Chapter 19, Kluwer Academic Publishers, Netherlands, pp: 409-437.

Sun, J.Z., 2008. Could Wood-Feeding Termites Provide Better Biofuels? In: Proceedings of the National Conference on Urban Entomology, Jones, S. (Ed.). Urban Pest Roundup, Tulsa, Oklahoma, pp: 50-54.

Sun, Y. and J. Cheng, 2002. Hydrolysis of lignocellulosic materials for ethanol production: A review. Bioresour. Technol., 83: 1-11.

Tartar, A., M.M. Wheeler, X. Zhou, M.R. Coy, D.G. Boucias and M.E. Scharf, 2009. Parallel metatranscriptome analyses of host and symbiont gene expression in the gut of the termite *Reticulitermes flavipes*. Biotechnol. Biofuels, Vol. 2. 10.1186/1754-6834-2-25

Tay, P.W., P.S. H'ng, K.L. Chin, L.J. Wong and A.C. Luqman, 2013. Effects of steeping variables and substrate mesh size on starch yield extracted from oil palm trunk. Ind. Crops Prod., 44: 240-245.

Tayasu, I., A. Sugimoto, E. Wada and T. Abe, 1994. Xylophagous termites depending on atmospheric nitrogen. Naturwissenschaften, 81: 229-231.

Tho, Y.P., 1992. Termites of Peninsular Malaysia. Forest Research Institute Malaysia, Kuala Lumpur Malaysia, ISBN-13: 9789839592146, pp: 22.

Tholen, A., B. Schink and A. Brune, 1997. The gut microflora of *Reticulitermes flavipes*, its relation to oxygen and evidence for oxygen-dependent acetogenesis by the most abundant *Enterococcus* sp. FEMS Microbiol. Ecol., 24: 137-149.

Tokuda, G., N. Lo, H. Watanabe, M. Slaytor, T. Matsumoto and H. Noda, 1999. Metazoan cellulase genes from termites: Intron/exon structures and sites of expression. Biochimica Biophysica Acta, 1447: 146-159.

Tokuda, G., H. Saito and H. Watanabe, 2002. A digestive beta-glucosidase from the salivary glands of the termite, *Neotermes koshunensis* (Shiraki): Distribution, characterization and isolation of its precursor cDNA by 5'-and 3'-RACE amplifications with degenerate primers. Insect Biochem. Mol. Biol., 32: 1681-1689.

Tokuda, G., N. Lo, H. Watanabe, G. Arakawa, T. Matsumoto and H. Noda, 2004. Major alteration of the expression site of endogenous cellulases in members of an apical termite lineage. Mol. Ecol., 13: 3219-3228.

Varma, A., B. Krishna Kolli, J. Paul, S. Saxena and H. Konig, 1994. Lignocellulose degradation by microorganisms from termite hills and termite guts: A survey on the present state of art. FEMS. Microbiol. Rev., 15: 9-28.

Warnecke, F., P. Luginbuhl, N. Ivanova, M. Ghassemian and T.H. Richardson *et al*., 2007. Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. Nature, 450: 560-565.

Watanabe, H., H. Noda, G. Tokuda and N. Lo, 1998. A cellulase gene of termite origin. Nature, 394: 330-331.

Wenzel, M., I. Schonig, M. Berchtold, P. Kampfer and H. Konig, 2002. Aerobic and facultatively anaerobic cellulolytic bacteria from the gut of the termite *Zootermopsis angusticollis*. J.Applied Microbiol., 92: 32-40.

Wilson, E.O., 1971. The Insect Societies. The Belknap Press of Harvard University Press, Cambridge, UK., ISBN: 978-0674454903, pp: 562.

Wood, T.G. and R.A. Johnson, 1986. The Biology, Physiology and Ecology of Termites. In: Economic Impact and Control of Social Insects, Vinson, S.B. (Ed.). Praeger, New York, USA., pp: 1-68.

Wood, T.G. and R.J. Thomas, 1989. The Mutualistic Association between Macrotermitinae and Termitomyces. In: Insect-Fungus Interactions, Wilding, N., N.M. Collins, P.M. Hammond and J.F. Weber (Eds.). Academic Press, London, UK., pp: 69-92.

Yeoh, B.H. and C.Y. Lee, 2007. Tunneling activity, wood consumption and survivorship of *Coptotermes gestroi*, *Coptotermes curvignathus* and *Coptotermes kalshoveni* (Isoptera: Rhinotermitidae) in the laboratory. Sociobiology, 50: 1087-1096.

Zheng, Y., Z. Pan and R. Zhang, 2009. Overview of biomass pretreatment for cellulosic ethanol production. Int. J. Agric. Biol. Eng., 2: 51-68.Zheng, Y., Z. Pan and R. Zhang, 2009. Overview of biomass pretreatment for cellulosic ethanol production. Int. J. Agric. Biol. Eng., 2: 51-68.