

## **INSECT ENZYMES FOR BIOTECHNOLOGY INDUSTRY: FROM NATURE TO INDUSTRIAL APPLICATIONS**

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### ***Abstract***

The demand for enzyme as biocatalyst is increasing for biotechnological based industry. This is due to their ability to catalyze specific biochemical reactions and able to generate products that have significant commercial value. Some of these enzymatic reactions cannot be replicated using conventional organic synthesis process. On the other hand, insects have evolved and established various enzymatic pathways that are not only important for their survival but also valuable. Therefore, scientists and engineers are searching a way to utilize insect's enzyme for industrial processing as an alternative for greener technology. A high-level idea about how the biological systems work, evolve and interconnect in nature is essential in order to put the application into the industry. A new field of innovation to increase performance and reliability, discoveries are in turn solving some of technology's greatest challenges as the demand for cleaner and greener technology is rising. This paper will review on the application of insect's enzyme as a biocatalyst in the field of biopharmaceutical, agricultural, food and pulp and paper industry.

***Keywords*** – Biocatalyst; biopharmaceutical; food; pulp and paper industry

## **ENZIM SERANGGA UNTUK INDUSTRI BIOTEKNOLOGI: DARIPADA ALAM SEMULA JADI KEPADA APLIKASI INDUSTRI**

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### **Abstrak**

Permintaan untuk enzim sebagai biokatalis meningkat untuk industri berasaskan bioteknologi. Ini disebabkan oleh kemampuan enzim untuk memangkin tindak balas biokimia tertentu yang dapat menjana produk yang mempunyai nilai komersil yang tinggi. Sebahagian reaksi enzimatik tidak dapat direplikasi menggunakan proses sintesis organik konvensional. Sebaliknya, serangga telah berkembang dan mempunyai pelbagai laluan enzimatik yang tidak hanya penting untuk kelangsungan hidup tetapi juga berharga untuk kepentingan industri bioteknologi. Oleh itu, saintis dan jurutera mencari cara menggunakan enzim serangga untuk pemprosesan perindustrian sebagai alternatif untuk teknologi hijau. Idea peringkat tinggi tentang bagaimana sistem biologi berfungsi, berkembang dan bersambung dalam alam semula jadi adalah penting untuk meletakkan aplikasi ke dalam industri. Bidang inovasi baru untuk meningkatkan prestasi dan kebolehpercayaan, penemuan itu seterusnya menyelesaikan beberapa cabaran teknologi paling besar memandangkan permintaan terhadap teknologi yang lebih bersih dan hijau semakin meningkat. Kertas kajian ini, mengkaji semula penggunaan enzim serangga sebagai pemangkin bio dalam bidang industri biopharmaceutical, pertanian, makanan dan pulpa dan kertas.

**Kata kunci** – Pemangkin bio; biofarma; industri makanan; pulpa dan industri pembuatan kertas.

## 1.0 INTRODUCTION

The demand for industrial enzymes is increasing in biotechnological based industry. This is due to their ability to catalyse specific biochemical reactions to produce certain products that have significant commercial value. Likewise, diverse enzymes possess by insects that have enabling them to utilize nearly different of organic matter as a food source also have significant commercial value. As insects have established diverse biological and chemical systems that might be useful to be applied into industrial application. These insect's enzymes are produced in three ways; partnerships with symbiotic microorganisms, obtaining from within their environment such as from nest or produced by the insects themselves. Enzymes which are produced and isolated from microorganisms are applied to speed up the process of conversion from one product into another product that have certain commercial value and this process known as biocatalysts. Whereby, the process that carried out by biocatalyst to generate chiral molecules is referred to biotransformation. The advantages of biocatalyst compared to conventional process, are lowering the operating temperatures, producing less toxic waste, emitting fewer greenhouse gasses and high yield. Whereas, product generated by biotransformation can be used as synthesis element in many of specialty molecules such as drug intermediates, bio-pesticide, preservative ingredients and chemicals manufacture.

Specialized insects have evolved enzymatic degradation pathways for almost all pesticides and plant toxins as well as for the destruction of pathogenic microorganisms which important for their survival. Insect hormones, pheromones, defence compounds and cuticle components are all synthesized with the assistance of specific and unique enzymes. Most of these reactions cannot be replicated using conventional organic synthesis techniques. Therefore, scientists and engineers are looking to higher-level ideas about how biological systems work, evolve and interconnect in nature. A new field of innovation to increase performance and reliability, discoveries are in turn solving some of technology's greatest challenges.as the demand for cleaner and greener technology is raising.

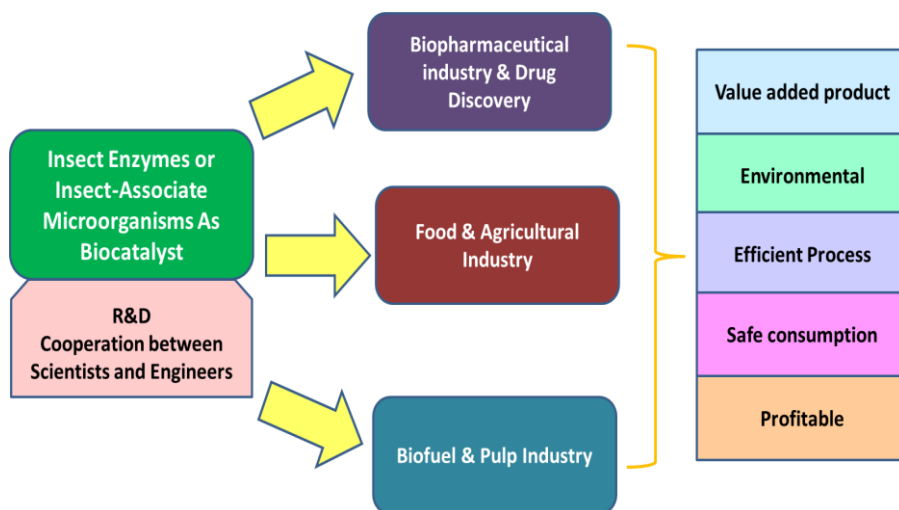


Figure 1: Overview on potential applications of insect enzymes and enzyme from insect-associate microorganisms in three major industries includes biopharmaceutical and drug discovery, food and agricultural industry and biofuel and pulp industry.

Biotechnology industry typically involves the conversion of complex organic material into much simpler products. For example, the conversion of carbohydrates polymers into much smaller carbon compound by glycosidase enzymes. According to Biotech Corp (Malaysian Biotechnology Cooperation, 2016), there are three policy treaties with industrial biotechnology sector that had been outlined in Malaysian Biotechnology Policy designed by the government. The emphasis of this policy is to encourage and support development prospects in the application of advanced bio-manufacturing and bio-processing technologies. Specific areas within the biotechnology sector were identified as potential growth areas in the policy and these include:

1. Bio-catalysts: Development of biocatalysts such as enzymes for food and feed preparations, bioremediation and other industrial processes.
2. Bio-processing: A growth area which can be applied in the production of biomaterials such as bioplastic, biofuel, fine and specialty chemicals such as cosmetic ingredients and electronic chemicals.
3. Bio-manufacturing: as applied to the development of bio-materials, enzymes (biocatalysts), biofuels, microbial technologies, biomass, biodegradable plastics, oleo chemicals and contract bio-process engineering.

## 2.0 BIOCATALYSIS FOR BIOPHARMACEUTICAL INDUSTRY

Enzymes are nature's way of generating the complexity of natural products and this can be used as powerful tool for drug discovery. Insect-derived enzymes such as cytochrome p450 and aldehyde oxidase are common enzymes that are used for synthesis of intermediate compound from the plant or insects' diet. The efficiency of metabolites product and enzymes manufactured by insects' symbionts microorganisms has been improved for over many years by natural selection (Berasategui et al. 2016) Interestingly, the intermediate compound generate by this symbiont have been tested for their toxicity and safety in eukaryotic host, hence create huge possibility of applications by humans (Berasategui et al. 2016). It is known that insects are able to metabolise xenobiotic compounds.

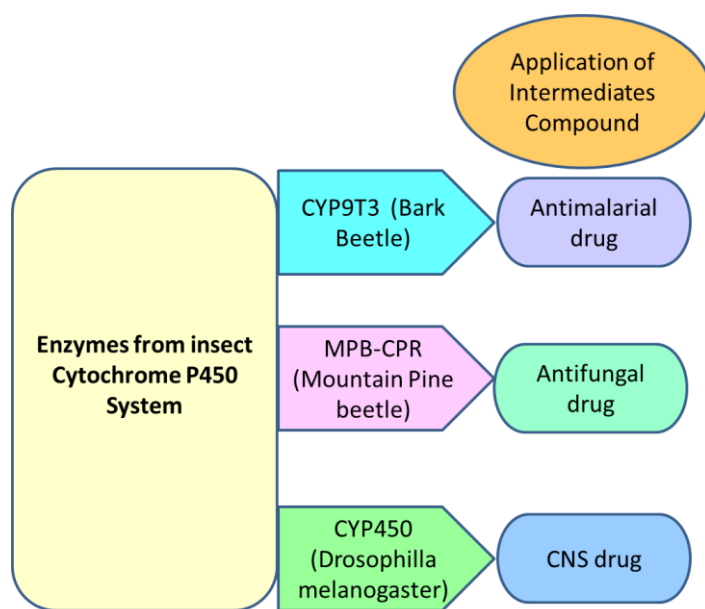


Figure 2: Illustration on insect enzymes cytochrome p450 and potential applications in pharmaceutical drugs discovery.

Cytochrome p450 from bark beetle (CYP9T3) catalyzes the hydroxylation of  $\beta$ -myrcene to form ipsdienol, a major component of the aggregation pheromone. The ipsdienol is an intermediate compound for antimalarial drug through biotransformation process using biocatalyst to synthesis polar antimalarial 1, 2, 4-trioxanes (Griesbeck et al. 2006). Remarkably, different cytochrome p450 from different organism can have different catalysis activity producing various intermediate compounds.

Cytochrome p450 from mountain pine beetle involve in biosynthesis of ergosterol and this compound is a target for antifungal drug (Dai et al. 2015).

Table 1: List of insects enzymes for biopharmaceutical application.

Enzymes	Source of enzymes	Application	Reference
Cytochrome-P450	- Drosophilla melanogaster (CYP450)	CNS drugs	Physiology n.d.
	- Eastern Ips pini (CYP9T3)	Antimalaria drug	Griesbeck et al. 2006
	- Mountain pine beetle (MPB-CPR)	Antifungal drugs	Dai et al. 2015
Aldehyde Oxidase:	- Drosophila melanogaster	Antibacterial agent	Sanoh et al. 2015, Pryde et al. 2010, Salhen 2016
	- Moth ( <i>Manduca sexta</i> ), cabbage armyworm ( <i>Mamestra brassicae</i> ),		
	- polyphemus silkworm ( <i>Antheraea polyphemus</i> )		
	- Domestic silkworm ( <i>Bombyx mori</i> )		
Ascorbate peroxidase	- <i>Helicoverpa zea</i> (corn earworm)	Antioxidant	Fountain et al. 2014
Protolytic enzymes	- <i>Eurygaster integriceps</i> (Sunn Pest)	Blood clothing medicine	Bandani et al. 2009

Also, insect's pheromone-degrading enzyme such as Aldehyde Oxidase (AO) is able to oxidize wide range of substrates related to pyrimidine and purine bases. AO is involved in the intermediary metabolism of many compounds has significant importance in studying drug metabolism and pharmacokinetics (Sanoh et al. 2015). Report by Pryde et al.(Pryde et al. 2010) suggested that the heterocycle oxidation by AO is very much relevance to drug discovery programs as AO is used to produce the active drug component in vivo. For example, biotransformation of AO to catalyse azetidiny ketolide analogue generate potent antibacterial agents with minimal hepatic turnover. Several insects include fruit fly, moth and silkworm have been studied for their AO capacity in generating intermediate compound for new drugs for various disease. Discovery of new biocatalyst from insect could potentially enhance finding of new drug for various diseases and infectious diseases.

### 3.0 BIOCATALYSIS FOR FOOD AND AGRICULTURAL INDUSTRY

Enzymes are used for wide range of applications: in bakery, cheese making, starch processing and production of fruit juices. Using enzymes can improve texture, nutritional value, and generate desirable flavours and aromas. Insects' enzymes or insect-associate microorganism provides opportunities to improve the process in food manufacturing. Important features such as food safety, food allergies and food nutrition have driven the search for enzymes with innovative biochemical characteristic to improve change the way the food is processed.

A rising number of gluten allergy consumers have focused the demand for gluten free food in the market. Grain pests such as wheat weevil, red flour beetle and grain beetles can produce various hydrolases to degrade carbohydrates and protein in their diet food (Mika et al. 2013). Studied by Mika et al. (Mika et al. 2013) showed that these pest have the ability to degrade or hydrolyse celiac disease-associate proteins (disease related to gluten allergy). The discovery of these gluten-degrading enzymes can be used as biocatalyst in manufacturing gluten-free food in food industry.

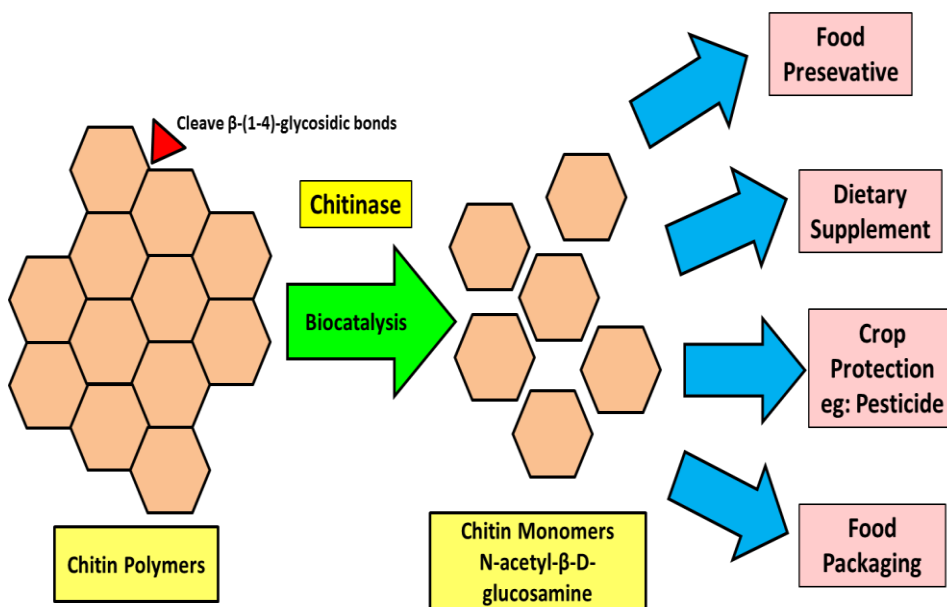


Figure 3: Overview on chitinase biocatalysis and potential industrial used.

Others biocatalyst such as chitinase has many roles in food preservativation and agricultural industry. Insect cuticles of an exoskeleton is built from chitin monomer, N-acetyl- $\beta$ -D-glucosamine and sclerotized proteins

(Merzendorfer & Zimoch 2003),(Stoykov et al. 2015). Chitin and chitosan are highly resistance against degradation hence this unique feature is suitable to slow down the growth of microbial pathogens in crop protection and food preservation (Muzzarelli et al. 2012). Chitinase is in remodeling chitinous structures by hydrolysing  $\beta$ -(1-4)-glycosidic bonds of chitin polymers to renovate chitin-containing raw material into biotechnologically functional components (Merzendorfer & Zimoch 2003). Furthermore, enzymatic hydrolysis of chitinase was found to be effective in the recovery of tannase enzyme which later producing gallic acid that can be used as preservative agent (Stoykov et al. 2015).

Insects' enzymes as biocatalyst not only applied to human but as well as to animal feedstock. Cellulolytic enzymes are well-studied in wood-feeding insects such as termites, red palm weevil and wood boring beetle as they are efficient in degrading the recalcitrant plant biomass such as lignin (Khiyami & Alyamani 2008; Scharf et al. 2011). Metabolisms of cellulolytic activity in insects occur, mainly the intestinal mucosa in the gut (Krishnan et al. 2014). Microorganism or gut symbiont in insects is known to facilitate the degradation of lignocellulosic compound by expressing different types of cellulase and hemicellulase enzymes (Krishnan et al. 2014). Improving feeding strategy by incorporating these microorganisms onto animal feed will improve the efficiency of feedstock's digestion systems by enhancing the bio catalysis of lignocellulosic (Wei et al. 2012; Beauchemin et al. 1997). This would allow the feedstock to get access for more nutrients and improving feedstock health and enhance meat quality thus increasing agricultural productivity (Andersen et al. 2005; Beauchemin et al. 1997).

Table 2: List of insect's enzymes for food and agricultural industry.

Enzymes	Source of enzymes	Application	Reference
Chitinase	<i>Manduca sexta</i> (tobacco hornworm)	Dietrary Supplement Preservative Crop protection	Stoykov et al. 2015, Muzzarelli et al. 2012, Hamid et al. 2013
Cellulase	<i>Bombyx mori</i> (silk worm)	Gluten free food Animal feedstock	Anand et al. 2010, Alarcon et al. 2015
Xylanase	<i>Rhynchophorus ferrugineus</i> (Red palm weevil)	Gluten free food Animal feedstock Food additive	Anand et al. 2010
Pectinase		Extraction juice from fruit	Anand et al. 2010



Protease	<i>Bombyx mori</i> (silk worm) <i>Rhynchophorus ferrugineus</i> (Red palm weevil) <i>Phthorimaea operculella</i> (Zeller)	Food additive Bakery	Anand et al. 2010, Alarcon et al. 2015, Mansouri et al. 2013
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#### 4.0 BIOCATALYSIS FOR BIOFUEL AND PULP INDUSTRY

In Asia, growth in the industrial biotechnology sector is being driven by the expansion of the biofuels industry in a number of Asian countries, particularly Malaysia, Indonesia, Thailand, India and the Philippines. The development of a national biofuels industry in these countries is strongly driven by the existence of significant quantities of feedstock in the countries and dedicated government support to foster the development of the industries. Malaysia is the second largest palm oil producer contributing 85% of the palm oil in worldwide market together with Indonesia (Kurnia et al. 2016). However, only 10% of oil palm is used whereas 90% of oil palm sector are considered as waste (This includes the empty fruit bunch, trunk and leaves) (Kurnia et al. 2016).

Major challenges in biofuel sector are the ability to fully utilise waste materials and able to convert the lignocellulosic material into biofuel. Biofuel production involves the breakdown of lignocellulosic materials (cellulose and hemicellulose) into simple sugar through saccharification process (Weng et al. 2008; Kurnia et al. 2016). However, phenolic polymer known as lignin become hindered to this process as it is highly resistant against degradation (Weng et al. 2008)(Wei et al. 2012). Lignin is the second abundant aromatic compound in plant cell wall that is wrapping around the cellulose and hemicellulose (Refer Figure 4) (Martínez et al. 2009). One of the sophisticated approaches to overcome this challenge is to use enzymes that capable of degrading lignocellulosic materials efficiently. Enzymes such as cellulase, hemicellulase, peroxidases and laccase have the capacity to randomly cut these polysaccharides. Wood-feeding insects such as termites and longhorn Asian beetle are also having the ability to degrade lignin (Scully et al. 2013; Geib, Filley, Hatcher, Hoover, Carlson, M. D. M. Jimenez-Gasco, et al. 2008). Likely, the majority of wood- feeding insects do have the capacity to degrade lignin as they consume lignin-rich lignocellulose materials as part of their diets. Researches in termites shows that the enzymes degrading lignin were highly expressed inside the midgut and the salivary by the symbiont microorganisms.

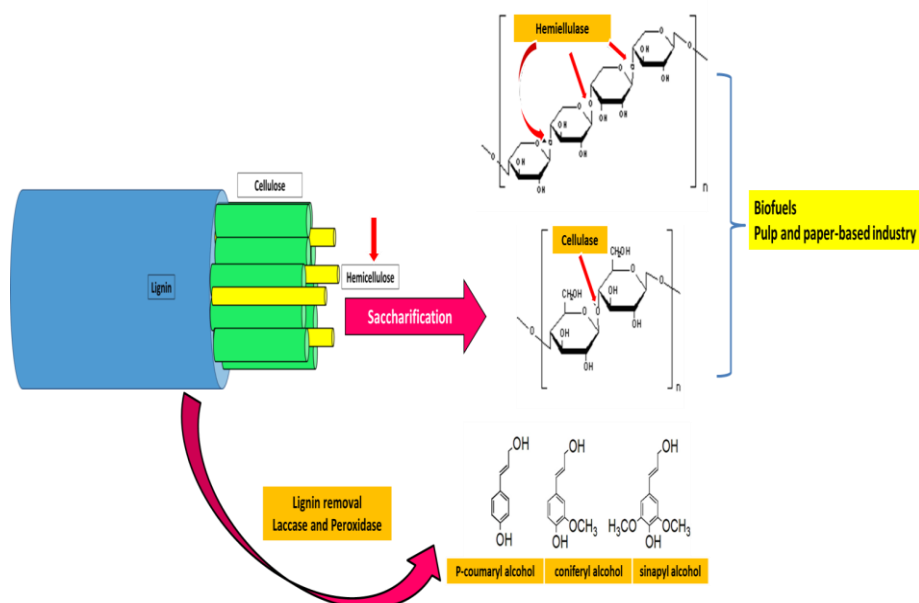


Figure 4: Illustration of lignin, cellulose and hemicellulose arrangement within the plant vessel tissue and saccharification process.

Table 3: Insect enzymes for biofuel and pulp industry

Enzymes	Source of enzyme	Application	Reference
Cellulase	<i>Oxya velox</i> (rice pest)		Shil et al.
- 1- $\beta$ Glucosidase	<i>Aularches miliaris</i>	Degrade or	2014; Scharf
- 1- $\alpha$ Glucosidase	(grasshopper)	modifying	et al. 2011;
Hemicellulase	<i>Reticulitermes flavipes</i>	cellulose and	Nakashima et
- Xylanase	(termites)	hemicellulose	al. 2002;
	<i>Coptotermus</i>		Kassim et al.
Laccase	<i>formosanus</i>	Modifying or	2016
	<i>Rhynchophorus</i>	degrade lignin	
Peroxidases	<i>ferrugineus</i>	Modifying or	
	(sago worm)	degrade lignin	
	<i>Eurycantha calcarata</i>		
	(walking stick)		
	<i>Bombyx mori</i> (silk		
	worm)		

Other wood feeding insect such as *R. ferrugineus* is famous for its damaging effect on many plants species including date palm and palm oil trees (Alarcon et al. 2015),(Riseh & Ghadamyari 2012). *R. ferrugineus* is a xylophagous insect which tend to have high amounts of plant phenolics and

tannins in their digestive tracts due to their diets (Alarcon et al. 2015),(Calderon Cortes et al. 2010). The ability of these larvae to consume variety of plant tissues provides opportunity to explore many potential enzymes that could potentially be useful in broad applications (Montagna et al. 2015). These unique enzymes act as biocatalyst to modify lignocellulosic component into biofuel and pulping process (Montagna et al. 2015; El-Mergawy & Al-Ajlan 2011; Wang et al. 2013). Insect gut of cellulase and xylanase are natural biocatalyst systems that are essential for biofuel production.

Current technologies used by pulp factory are inefficient in removing the lignin as this compound are resistant to degradation. In fact, in industrial process large amount of the cost are derived from lignin removal process; approximately between 160 - 200 million dollars per year depending on types of treatment used in order to produce high-quality fibre (Novaes et al. 2010). Enzymatic pulping involved the use of enzymes as pre-treatment to replace or reduced the amount of chemical used during pulping process. An enzyme is a protein that acts as a biological catalyst to carry out specific metabolic chemical reactions in living organisms(Bajpai 2009). Recently, a lot of studies have been conducted to integrate the use of enzymes as a pre-treatment before subjected to either chemical (bio-chemical) or mechanical (bio-mechanical) pulping (Abdel-Hamid et al. 2013; Bholay et al. 2012; Waung & Chemistry 2010). In pulping process, the raw material was first treated with enzymes such as laccases or peroxidases to remove lignin and enhanced the efficiency of later pulping process (Zucca et al. 2014; Abdel-Hamid et al. 2013). Incorporating enzymatic pre-treatment has shown to reduce the amount of chemical usage in chemical pulping (Wang et al. 2013; Gavrilescu & Chisti 2005; Miguel & Santos 2014). For example, hemicellulase enzymes such as xylanase can enhance the bleaching efficacy allowing a dramatic reduction in the consumption of chlorine (Thakur et al. 2012). This is because less chemical is required to degrade lignin, allowing more access to cellulose and hemicellulose (Wang et al. 2013; Gavrilescu & Chisti 2005; Miguel & Santos 2014). Also reduce the chemical for bleaching to remove the brownish colour that is due to presence of lignin (Eriksson & Bermek 2009; Wang et al. 2013).The transition of conventional pulping processing into a modern bioprocessing by integrating with the use of enzymes are believe to improve the ability to fully utilize all components of substrates to produce high quality fibres and other potential industries.

## **5.0 FUTURE PERSPECTIVE**

Realising the importance to preserve the environment at the same time having an efficient processing has prevailed on adaptation of natural processing into industrial application. Integrating the use of insect enzymes as biocatalyst would reduce the production of harmful pollutant and less emission of greenhouse gasses as the process are more efficient and moderate chemical usage. The

explorations of insect's enzymes have given an opportunity to enhance the industrial processes that are more economical and environmental friendly, hence promoting greener technology.

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