Small Review: Strategies for Palm Kernel Cake (PKC) As a New Potential Substrate in Biofuel Production

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Abstract—The economic dependency on fossil fuels and the resulting effects on climate and environment have put tremendous focus on utilizing fermentable sugars from lignocellulose, the largest known renewable carbohydrate source. Palm kernel cake (PKC) is a residue from palm oil extraction presently only used as a low protein feed supplement. It's contains 50% fermentable hexose sugars present in the form of glucan and mainly galactomannan. This makes PKC an interesting feedstock for processing into biofuel or in other biorefinery processes. This article reviews biotechnological innovation on Palm Kernel Cake (PKC) as new potential of fermentable sugar for biofuel production. Strategies for biofuel production by utilizing palm kernel cake by several pretreatment processes to convert glucan and especially galactomanan into fermentable hexose sugar and further requirements to make fermentative biofuel production a successful industrial process are also discussed. This material recovery especially from lignocellulose agricultural wastes by product of palm oil mill industry into this potential bioproducts has not only benefited in oil palm planted but also to the environment and helps preserve natural resource.

Keywords- Palm Kernel Cake; Biofuel; Lignocelluloses; Fermentable Sugar; Pre-treatment

I. INTRODUCTION

Increasing the global awareness about the environment, growing energy demand, and fluctuating oil prices has attract the attention of scientific and local community towards production of biofuels from biomass. Only biomass has the potential to replace the supply of an energy hungry civilization. Plant biomass is an abundant and renewable source of energy-rich carbohydrates which can be efficiently converted by microbes into biofuels via fermentation process.

Over the last 4 decades, the palm (*Elaeis guineensis*) oil industry in Malaysia has grown tremendously. In 2012, Malaysia has 5.08 million hectares of land that are planted with oil palm trees and contributes 39% of the world's total

palm oil production and 44% of world exports an increase of 1.5% against 5.00 million hectares recorded in 2011.Malaysia produced 18.79 million tonnes of crude palm oil in 2012 and it has forecasted a steady production for the next decade. With the immense scale of the industry, abundant quantity of oil palm biomass is generated and it is commonly left to decompose in the surrounding area around mills while only a small amount of this biomass is used to generate steam and electricity. There are approximate 400 palm oil mills in Malaysia generate about 95 million tonnes of biomass in 2012 [1].

Raw biomass is valueless and contributes to the environmental degradation problem if it is not handled carefully. Elimination of large-scale accumulation of biomass with a another technology to value added for this biomass now became the popular strategy to some country that can worth for billions of hollars. For example, the oil paim biomass can be converted into fertilizer, composite Ancerian matters Slinge medium density fiberboard, molded wares, paper and pulp. The used of Palm Kernel Cake (PKC), a solid residue biomass from palm oil mill **interfination neeCaltenaogy** feed stock which is domestically available, renewable and environmentally friendly in the biofuel world production become a promising technology. Therefore, in this review paper, Palm Kernal Cake (PKC) has been investigated as a new another potential substrate that is available in vast quantity which can be converted for the biofuel production and some strategies can be conducted to allow this substrate became as a another good source of energy.

II. PALM KERNEL CAKE (PKC)

Over the last decade, demand for palm (*Elaeis guineensis*) oil has grown by on average 2.3 million tonnes annually as one of the largest sources of cooking oil in the world [2] has supplied 62% of the palm oil needed to meet this demand, whereas Malaysia has supplied 30% and other countries 8%. From the period of March 2008 to 2009, world annual production of palm oil exceeds 43 million metric tonnes [3] and this trend is set to be continue as total palm oil demand is projected to rise by another 5 million tonnes annually by 2015[4].

Palm kernel cake (PKC), one of the major agro-industrial residue solid waste by-products of the palm (*Elaeis guineensis*) oil processing industries in Malaysia, Thailand and Indonesia. Malaysia is the world's second top supplier of palm oil after Indonesia with a total production approximately 85% for these two countries. PKC that are produced annually after the oil extraction process of palm kernels can exist into two types, palm kernel meal (PKM) or palm kernel expeller (PKE) depending on the method used for the extraction of the oil from the kernel 'with the latter normally containing slightly higher oil content' [5]. Based on current production of palm oil, it can be estimated that around 4.5 million tons of PKC are available, mainly in Malaysia and Indonesia [6].

In 2001, 11.8 million tonnes of crude palm oil which consist of 3.3 million tonnes of palm kernel, 1.5 million tonnes of crude palm kernel oil and 1.7 million tonnes of PKC was produced in Malaysia [7] and 1.9 million tonnes of PKC produced in 2003-2004 [8]. This numbers was further increase in 2006 to 2009 when Malaysia itself produced 2.20 million tonnes [9] and 2.31 million tones [10] of PKC respectively and exported 2.12 million tonnes of PKC in 2006 of it without any further application here except as animal feed. Furthermore, Indonesian palm oil production will continue grow and will reach 25.4 million metric tons (mmt) in marketing year (MY) 2011/2012, due to expanded harvested areas and higher yields.

Exports of PKC itself increased by 10.4% to 2.46 million tonnes by 2012 to Europe with 0.96 million tonnes (or 38.9% of total palm kernel cake exports, New Zealand 0.70

million tonnes (28.6%) and South Korea **06**73**million** (19.1%), the major Malaysia PKC export markets [1] ISSN: 2088-5334

Due to the large amounts of palm fruit produced and being processed annually plus accumulation of PKC as a residue solid wastes, it is important to optimize the use of the by-products or waste streams generated [12]. Approximately, 6 tonnes of waste palm fronds, 5 tonnes of empty fruit bunches, 1 tonne of palm trunks, 1 tonne of press fibre (from the mesocarp), 500 kg of palm kernel endocarp, 250 kg of palm kernel press cake, and 100 tonnes of palm oil mill effluent (POME) can be obtained from each tonne of crude palm oil produced[13] from the fresh fruit bunches after the oil extraction. These amounts are significant enough to consider PKC as bioresource of a new potential raw materials for many industries especially in biofuel industries production because it has been suggested that residues such as empty fruit bunches and palm press fiber can be used as solid fuel for incineration or converted into various liquid biofuels [14].

Furthermore, from the view point of logistics and cost, Palm Kernel Cake (PKC) biomass offers the best prospects for commercial exploitation especially as a feed stock for biofuel production. The uses of PKC in these sectors will not only provide additional revenue to the industry but will also help in achieving the industry vision towards a 'zero waste' economical technology strategy in the long-term and environmentally friendly industrial in the utility systems.

III. CURRENT APPLICATIONS OF PALM KERNEL CAKE (PKC)

Currently, most of the PKC produced after an extraction of oil palm from palm oil seeds in Malaysia is exported at a low price to Europe for use as cattle feed concentrates in dairy cows. PKC is an established feed ingredient or supplement for ruminants, supplying valuable dietary sources of protein, energy and fibre. PKC has also been successfully tested in poultry and swine feeds at low levels of incorporation due to its high fibre and low protein content [15,16]. The low cost and availability of PKC in many tropical countries where aquaculture is practiced have recently generated much interest in its potential use in fish diets but it is very little information is currently available on the use of PKC in fish diets.

In Malaysia, PKC that is consist of 15% protein [12] and 30% of cellulose roughly [17] is normally used as dairy, swine, aquaculture and ruminants low protein feed [19-21]. High indigestible fibre content, poor amino acid balance, with lysine being a major limiting amino acid and low concentrations of relatively low nutritional value protein has limit the use and efficiency of PKC as feed, especially in broiler feeds[17]. The indigestible portions in PKC are actually a non-starch polysaccharides (NSP) that is consist of 78% β-mannan, 12% cellulose, 3% arabinoxylans, and 3% glucoxylans. water-insoluble These unfavorable characteristics in PKC causing low palatability and indigestion to poultry. As a result, the inclusion of PKC in

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Carbohydrates source can be found abundantly mostly in the structural parts of plans like leaves and stems that commonly known as lignocelluloses, which consist mainly of cellulose, hemicelluloses and lignin. In many studies, the content of total carbohydrates in PKC has been determined to approximately 50% [22-24]. The majority of glucose in PKC has previously been verified not to be starch but rather cellulose [23,25]. Mannan is main polysaccharides that are largely available in PKC cell wall that is constituting 35.2% on dry weight which are content of water insoluble glucomannan and small amount of water soluble galactomannan thus making it resemble very much cellulose by crystalline, hard and water insoluble[25,17]. Mannose is the principal carbohydrate (neutral sugar) present in palm oil kernels with a content in the range of 30-35%, but also 7-9% glucose is present [23,26,27]. The main component and composition (% on dry basis) of the PKC with a sugar reported as anhydrous form [28] are listed in Table 1

TABLE 1 COMPOSITION OF PKC

Component	Composition (%)
Glucose	7.7
Xylose	2.6
Arabinose	1.1
Galactose	1.9
Mannose	35.2
Protein	15.0
Lignin	15.1
Ash	5.0

In terms of fibre content, the fibre in PKC is mainly available as hemicelluloses that are consisting of 58% mannans [29], moderate amounts of cellulose and small amount of other polysaccharides [30]. The glucan content was 8% and the remaining sugars accounted for less than 6%. The oil/fat content in PKC is around 9%. This value is accordance with what has been published and much depending on the production method [31, 32]. The high content of polysaccharides in PKC and the favourable composition of the sugars with a high percentage of fermentable hexose (C6) sugars make it a potential raw material for biofuel production [28].

V. MANNAN IN PAL KERNEL CAKE (PKC)

Mannan or galactomannan is the main carbohydrate in PKC. Galactomannan consists of a linear $(1\rightarrow 4)$ linked β -D-mannopyranose backbone and small amount of $(1\rightarrow 6)$ linked α -D-galactopyranose (galactomannan) side groups [33, 34, 27]. However, the galactose substitution is low (12-20%) [27,34]. Mannan in PKC resembles in very much cellulose by being crystalline hard structure and water insoluble due to the low substitution[33,35,36] is fairly easy

to digest by endo mannanases enzymes accessible to enzymes compared with cellulose [37]. In addition to mannans, PKC also contains some cellulose and low amounts of 4-O-methyl-glucoronoxylan and arabino xylan[35].

VI. STRATEGY OF USING PLM KERNEL CAKE (PKC) FOR BIOFUEL PRODUCTION

The critical requirement for enhancing PKC before being used as a core substrate for biomass-to-biofuel processes is their physic-chemical pretreatment for enhancing and improved the accessibility of PKC substrate to enzymatic attack by biofuel producing microorganism. Many biomass process methods have been developed and yet much of the PKC as a study and researches still devoted to cellulose production or improvement of PKC content as animal feed. One of the important strategy and become first requirements that's needed in the utilization of PKC for the production of biofuel is to study how efficient the PKC to produce a fermentable hydrolysate rich in glucose and others fermentable sugars like mannose, xylose, and galactose.

Two main strategies methods to overcome this limitation are:

(i) Pre-treatment of PKC

Non-starch polysaccharides such as cellulose are often difficult to process into fermentable sugars. Usually, the process involves an extensive thermal/chemical pretreatment prior to enzymatic hydrolysis and fermentation [38].

(ii) Hydrolysis Process of PKC to Monosaccaharides

The use of fungi (particularly *Aspergillus sp.*) in solid state fermentation (SSF) and commercial enzymes to breakdown and reduce the hemicelluloses, cellulose and lignin in PKC to simple fermentable sugars.

i. Pretreatment of Palm Kernel Cake (PKC)

The main goal of pre-treatment process for several cellulosic and lignocelluloses materials in biofuel production is to make sure this kind of materials are open up and make it accessible prior hydrolysis for conversion to fuels for the enzymes. These pre-treatment processes normally change the physical and chemical structure of the cellulosic and lignocelluloses biomass that can improve the hydrolysis rates during the hydrolysis process. During the past few years a large number of pre-treatment methods have been developed, including acid and alkali treatment, autoclaving (high temperature pre-treatment) ammonia explosion, and others. Many methods have been shown to result in high sugar yields, above 90% of the theoretical yield for lignocelluloses biomass such as woods, grasses, corn, and so on.

And view of the range of 2 5% [38, 40] at high temperature (160-239°C) and pressures (10 atm 41] and high temperature production of the range of 2 5% [38, 40] at high temperature (160-239°C) and pressures (10 atm 41] and high temperature production of the range of 2 5% [38, 40] at high temperature production of the range of 2 5% [38, 40] at high temperature (160-239°C) and pressures (10 atm 41] and high temperature production of the range of 2 5% [38, 40] at high temperature (160-239°C) and pressures (10 atm 41] and high temperature production of the range of 2 5% [38, 40] at high temperature (160-239°C) and pressures (10 atm 41] and high temperature (160-239°C) and pressures (10 atm 41] and high temperature (160-239°C) and pressures (10 atm 41] and high temperature (160-239°C) atm 410 a

Study by [42] indicated that high temperature pretreatment by just a simple autoclavation at 126 °C for 11 min can released 72% mannose and 15% glucose. It was realize that the processing step like with heating of the oil palm fruit/kernel, such as steam sterilization, digestion, pressing and kernel separation [43] during the production of palm kernel oil might act as a pre-treatment of PKC where it likely to alter the structure and convertibility of PKC. Using the autoclavation method appeared to be a good trade off between enhancing the accessibility of the PKC (mainly mannan) for enzymes and to avoid too high sugar loss. Furthermore, the autoclavation functioned as a sterilisation thereby minimised the risk of microbial contamination.

Recent laboratory work by [26] indicates that a high temperature pretreatment similar to what is used for lignocellulosic materials is not a prerequisite for enzymatic hydrolysis of PKC. The ability to enzymatically hydrolyze PKC without a high-temperature pretreatment step is an advantage since it significantly reduces cost [44] and makes the process more economically feasible. Another advantage is that the protein in PKC, which makes the residues valuable as a feed product, is preserved. In addition, the absence of a pretreatment process avoids the potential formation of degradation products such as acetic acid, furans, and phenolic compounds [37, 45, 37]. These inhibitors are known to affect the fermentation efficiency of ethanol-producing microorganisms such as *S. cerevisiae* [46,47,48].

ii. Hydrolysis of PKC into Monosaccharide's

a) Microbial Degradation of PKC

Polysaccharides such as starch, cellulose and other glucans, pectins, xylans, mannans, and fructans are present as major structural and storage materials in such a lignocellulosic material. These constituents may be degraded and modified by endogenous enzymes from certain microorganism. There are many microorganisms are capable of decomposing cellulose and mannans. Enzymes from fungi such as Aspergillus niger [49], Trichoderma reesei [50] and Sclerotium rolfsii [51] deserve the most attention to decomposing this kinds of lignocellulosic materials that is consist of cellulose, hemicelluloses and lignin . In nature, lignocellulolytic microbes interact in mixed culture to degrade lignocellulose e.g., wood decay [52]. Moist solid substrate in the absence of free water in Solid substrate fermentation (SSF) resembling the natural habitat of filmentous fungus that is normally growing on solid lignocellulosic materials [53].

Limited studies reporting the effect **vforgypertsequeet** on content of reducing sugars in PKC except Ng et al. [54] reported that enzyme treated and fungal remented Palm Kernel Meal (PKM) increased reducing sugars content by about 69% mannose (from 2.87 to 9.25 mg/g) and 65% glucose (from 2.87 to 8.09 mg/g), respectively. The hydrolysis of insoluble cellulose and hemicellulose into soluble sugars indicate an increase in usable energy in the enzyme treated PKC for monogastric animals such as chickens compared to the untreated PKC [54].

Studied has been done on the application of *Aspergillus flavus* as isolated fungus from local PKC to increase the mannose content of PKC via the degradation of β -mannan in PKC shows that the optimum reducing sugar produced in novel laterally aerated moving bed (LAMB) bioreactor was 79.61 mg mannose g-1 dry PKC at 96 h of fermentation time, as compared to 90.91 mg mannose g-1 dry PKC at 120 h in the Erlenmeyer flask [55, 56]. On the bases on this results, it is found that the fungal enzyme treated PKC reduced crude fiber (CF), hemicelluloses and cellulose contents resulted in increased CP and soluble sugar (glucose, fructose, galactose and sucrose). The significant reduction in hemicelluloses content indicates that the enzyme is effective in breaking down mannan-hemicellulose, the main component of the fiber in PKC [39,57].

The use of microbial degradation of lignocelluloses materials to increase feedstock digestibility has several advantages that can gave an economical saving like low energy inputs, modest hardware demand, and very environmental friendly because of no hazardous chemicals and condition applied and no damaging product generated during the degradation process [58].

b) Enzymatic Hydrolysis of Palm Kernel Cake (PKC)

Mannan is an important component in PKC that can be classified in four subfamilies which are linear mannan, glucomannan, galactomannan, and galactoglucomanan. Each of these polysaccharides subfamilies, it presents a β-1, 4linked backbone containing mannose or a combination of glucose and mannose residues [59]. Enzymatic hydrolysis is the most common method of producing biofuel from lignocellulosic biomasses. Hydrolysis of this component in PKC into fermentable sugars requires a numbers of different enzymes especially for the degradation of mannan and it requires longer retention times. The endo- β -D-mannanase (E.C 3.2.1.78, mannan endo-1,4- β-D-mannosidase) is an enzyme that randomly cleave within the 1,4- β –D-mannan chain of galactomannan, glucomannan, galacto glucomannan and mannan [60] and oligosaccharides in PKC can be further cleaved by β -Dmannosidase, β -D-glucosidase, and α -Dgalactosidase to produce mannose, glucose and galactose [61].

As illustrated in Figure 1, mannan-degrading enzymes are composed of β - mannanase (1,4- β -D-mannan mannohydrolase, EC 3.2.1.78), β -mannosidase (1,4- β -Dmannopyranoside hydrolase, EC 3.2.1.25), and β glucosidase (1,4- β -D-glucoside glucohydrolase, EC **Activities of the second sec**

1,4-linked internal linkages of the mannan backbone randomly to produce new chain ends. The degradation of galactomannan and galactoglucomannan by β -mannanase is greatly affected by the extent and pattern of substitution of the mannan backbone [59].



Fig. 1 Enzymatic attack on galacto glucomannan structure [59]

Enzymatic hydrolysis of mannan or galactomannan requires the action of endo-1,4- β -mannosidases (E.C. 3.2.1.78), β-mannosidases (E.C. 3.2.1.25) and 1,6-α-Dgalactosidases (E.C. 3.2.1.22) for removal of the galactose side groups. Cellulose can behydrolysed by cellobio hydrolases (EC3.2.1.91), endo-1,4- β -d-glucanases (EC 3.2.1.4) and 1,4- β -dglucosidases(EC 3.2.1.21). The xylan backbone is hydrolysed byendo-1,4- -d-xylanases (EC 3.2.1.8) and 1,4-β-d-xylosidases (EC3.2.1.37) and removal ofside groups requires the action of α-larabinofuranosidases (EC 3.2.1.55) and α -glucuronidases (EC3.2.1.139). Efficiently releasing all fermentable sugars in PKC therefore requires the combined action of a large number of different enzymes. In addition, some of these enzymes could works synergistically and increase overall amount of monosaccharides released. Because of its complex chemical structure, the fiber of PKC requires a enzymes including mannosidases, combination of galactosidases, glucosidases and xylanases to release the potential fermentable sugars to be of use for biofuel production. The lignin content in PKC was only 15%, which is lower than some other lignocellulosic materials such as wood or straw [62] and this low lignin content is generally [63]. It is generally believed that the lignin is from shell fragments present in the PKC [64].

A lot of study has been done by many researchers to improved the nutritive value of PKC for the use of animal feed using a mannanase, galactosidase and cellulose. The same strategy can be used for biofuel production because the used of this kind of enzyme will improved the fermentable sugar content in PKC. Sundu B and Dingle (2003) [65] reported that mannose content increased 45 folds from 5.38 mg/g dry matter (DM) PKC to 243.4 mg/g, glucose (from 5.99 to 221.25 mg/g), galactose (from 5.44 to 2.266 mg/g) and xylose (from 5.07 to 219.58 mg/g) when it treated by enzymes. The data clearly suggests that the enzyme effectively hydrolyzed the lignocelluloses in PKC to simple sugars which resulted in an increase in the total reducing sugar (glucose, mannose, galactose and xylose) contents by proximately 200 folds. The results in agreement with the result study by Saenphoom, et. al (2011), that also proved that exogeneous enzymes used during the hydrolysis can give a significant increase up to 200 fold in the total reducing sugars measured in the treated samples compared to the control, suggesting the effective hydrolysis of structural carbohydrates (hemicelluloses and celluloses) into monosaccharide sugars.

Hydrolysis of PKC using a mixture of endomannanase, β -mannosidase, and a cellulose enzymes preparation with a volume ratio of 30:10:1 for 192 hr of hydrolysis time, and using the highest enzyme loading, it was possible to reach 88% conversion of mannan into mannose and 69% of the glucan/cellulose into glucose. This corresponded to a final concentration of 142 g/kg of mannose and 21 g/kg of glucose (concentrations are reported as grams per kilogram of slurry and not liter due to the high content of solids. In total, the amount of fermentable hexose sugars corresponded to 467 g/kg PKC [26].

The requirements for pretreatment has been investigated by [42] also has proved that enzymatic hydrolysis of polysaccharides from the cell-wall material present in PKC to obtain monosaccharides PKC using Mannaway 25L as the best enzyme for realizing mannose and Gammanase 1.0L worked well in degrading cellulose and mannose. Binary mixtures of Mannaway 25L and Gammanase 1.0L in a ratio of 1:1 showed good synergistic effect releasing 30% more mannose than the sum obtained using these enzymes individually. Using an enzyme loading of 2.3 mg protein/g PKC resulted in 63% of mannan in PKC being hydrolysed to mannose in 24 h, and in 96 hr of hydrolysis time, a total of 365 g mannose and glucose could be produced per kg PKC. The hydrolysis tests by several researcher showed that the yield of monosaccharides obtained represented nearly 75% of the total polysaccharides content in PKC [42]. It shows that PKC was demonstrated to be a potentially good raw material for biofuel production.

VII. PALM KERNEL CAKE (PKC) FERMENTAION FOR BIOETHANOL PRODUCTION.

Biofuel produced by fermentation of carbohydrates is increasingly used as fuel in the transportation sector. It is a promising substitute to fossil fuels due to the carbon dioxide neutrality and the fact that it can be a domestic renewable energy source. The raw materials used for industrial bioethanol production are currently sugar cane or starch containing materials such as corn or grain. However, as a result of the growing demand for bioethanol world wide, there is an interest to look for alternative sources of fermentable carbohydrates. **International formentation is a flassic** fermentation where it shows the ability of cells to absorb glucose or other **Action absorb glucose** or **Action absorb glucose** or **Action absorb glucose** or

It has been reported than PKC can be used as bioethanol production. PKC with a high content of hexose sugars (50% of total weight) after the enzymatic hydrolysis that could produced 200g ethnol/kg PKC from 467g/kg PKC [26] with the medium enzyme loading and 35% of solid concentration to obtain 84% conversion of mannan and glucan in 216 h of fermentation hour using a Solid State Fermentation (SSF) process. Under these conditions and without addition of any nutrients, the sugars were readily metabolized by traditional yeast S. cerevisiae with average fermentations yields of 0.43±0.02 g/g. The ability S. cerevisiae as fermenting organism to utilize PKC for ethanol production using only modest process conditions (low temperatures) and it is advantageous since it preserve the protein in the solid residues, which could be used as a high-value feed product [26].

The rationale behind seeking alternative and new feed stocks for ethanol production is continued increase in energy demand worldwide. The use of PKC as an alternative feed stock for ethanol production holds great promise due to its widespread availability and abundance.

VIII. CONCLUDING REMARKS

The palm oil industry is a Malaysian success story and has been the backbone of Malaysia's social and economic development. Sustaining its competitive edge remains the most important challenge for the industry especially to improve the efficiency and productivity to reduce cost. Utilization of residue solid wastes by product generated is an innovative marketing approaches to explore opportunities to diversity the income base. Biotechnology must, therefore been apply in the industry in order to achieved this mission.

Successful fermentation of the mannose, glucose and others fermentable sugars from PKC for biofuel production obtained using traditional yeast *S. cerevisiae* without any use of additional nutrients shows that the carbohydrates in PKC could efficiently be utilized for ethanol production. From the palm oil fruit it is therefore possible to produce liquid fuel in the form of biodiesel (from the oil), bioethanol and a protein rich animal feed from the fermented PKC residue. This renewable energy from biomass will become one of the most important sources of energy in the future. Research and development (R&D) in this area will provide (2013) and be solution for the industry in order to meet the increasing of energy demand and this is the beginning of a new industry the 'Oil Palm Biomass Industry' to Malaysia Palm Oil Mill Industry.

ACKNOWLEDGMENT

Authors want to acknowledge Universiti Kebangsaan Malaysia for the research on Palm Kernel Cake (PKC) as potential substrate for biofuel production at the Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment.

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