Poster:

Fungal bioconversion of old oil-palm trunks by enzymatic hydrolysis on development of alternate energy source

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Abstract The progressive depletion of fossil fuels has been causing increasing concern on rising energy consumption and environmental issues, such as greenhouse gas emission (GHG) and global warming. Due to low productivity of oil-palm tree after 20 - 25 years, the trees passed their economic age followed by the cutting-activity for replanting. Consequently, the old oil-palm trunks are one of the most abundantly available renewable resources produced, especially by Indonesia and Malaysia. We found that the felled oil palm trunk contains large quantity of sap with high concentration of free sugar contents. The oil-palm trunk residues which are the residual substances after squeezing sap will be discharged in large quantity. Composition analysis revealed that oil-palm residues mainly consisted of 73.12% holocellulose (cellulose and hemicellulose) and 24.6% of lignin. We tried the screening of filamentous fungus which can produce high-activity enzyme against oil-palm trunk residues as feedstock of bioethanol production. A filamentous fungus, which is *Penicillium rolfsii* with strong activity against oil-palm trunk residues was selected for saccharification experimental study. The result showed that higher amount of sugar production was achieved comparing to the commercial enzymes (Celluclast 1.5L and Accellerase®1500) on hydrolysis of oil palm residues, which is 1 to 2-fold of higher activity. Hence, *Penicillium rolfsii* have attracted a great deal of interest as oil-palm residues degrader due to their superiority activity against commercial enzymes.

Keywords*: Penicillium rolfsii*, oil-palm residues, saccharification, commercial enzymes.

Introduction

Indonesia and Malaysia together account for 88 percent of the world's supply (Sumathi *et al*., 2008). The most significant attribute of oil palm trees is their high productivity. About 3.4 to 4.9 tons of palm oil are produced per hectare per year. This is 10 times the amount of soybean oil. The productivity is extremely high. However, the productivity of the trunks generally diminishes after about 25 years. At that point, they are cut down and left in fields. In some cases, the trees are withered by injecting chemicals into their roots, and this is a potential cause of soil pollution. In Malaysia, about twenty to thirty million tons of old trees are cut down annually. Presently, there are no uses for these old trees. The outer layers of some of them are removed and used as plywood, but almost of tree are discarded. In the previous study, an ethanol production system has been developed using the sap from felled-down trunks of old oil palm trees (Yamada *et al*., 2010). Yamada *et al*., 2010 reported that a significant increase of fermentable sugars in oil-palm sap occurs during storage of the trunks after logging to reach the concentrations comparable to that of sugar cane juice, indicating the old and felled oil palm trunks are promising feedstock. A great deal of palm residues remained after the sap has been extracted by development of a sap squeezing system. For this reason, the lignocellulosic oil-palm trunk is available all year around becomes promising resource which for bioethanol production.

Due to the renewable and ubiquitous nature of lignocellulosic biomass and its noncompetitiveness with food crops, production of ethanol from lignocellulosic biomass seems very attractive and put a tremendous amount of effort into the research aspect for bioconversion. Generally, bioethanol converted from edible food resources, such as corns and sugarcanes by fermenting sugar is called first-generation biofuels (Goh *et al*., 2010, Singh *et al*., 2011). In this respect, the search for non-edible biomass such as oil-palm trunk as agricultural residues for production of biofuels is favoured to avoid confliction with food. Hence, oil palm trunk residues was used as sustainable lignocelluloisc biomass to produce reducing sugars prior to production of alternate energy source, such as biofuels or other value-added chemicals in order to bring the world for second-generation biofuels. A fungal isolate, namely *Penicillium rolfsii* was selected in this study due to its ability to produce a highly hydrolysis efficiency of fungal enzymes to degrade oil palm trunk residues comparing to the commercial enzymes (Celluclast 1.5L and Accellerase®1500, which is 1 to 2-fold of higher activity.

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Materials and Methods

Preparation of oil-palm trunk residues

Oil palm residue which mainly obtained from the trunks of palm tree was used as a solid substrate. The oil-palm trunks which were cut in cubes dried in oven at 80°C. The cubes were ground finely by using Variable Speed Rotor Mill "pulverisette 14" (Fritsch GmbH, Germany), with a sieve insert 0.5 mm. Oil-palm residues were used for experimental study by removing its starch content (destarched-oil-palm residue). Starch content of oil-palm residues was removed by using α-Amylase from *Bacillus amyloliquefaciens* and amyloglucosidase was added by incubation temperature of 60°C for overnight. After incubation, oil-palm residues were washed for several times by using distilled water and dried in oven at 70°C. The total sugar content of each sample was determined via enzymatic hydrolysis of starch into glucose, using Total Starch Kit (Megazyme, International Ireland, Wicklow, Ireland). The composition content of oil-palm residues was determined by Toray Techno Co. Ltd., Japan.

Fungal growth and storage

The previously isolated *Penicillium rolfsii* was subsequently grown on PDA plates and also in minimal salts medium containing 1.0% of oil-palm trunk residues. Pure culturing of the fungus was done on 14 days interval from the working culture plates. Agar blocks containing the fungus were placed in sterile distilled water and stored at 4°C.

Total Sugar Determination by Phenol-Sulphuric Acid Method

Oil-palm residues (100 mg) were hydrolyzed with 1 ml 72% (w/w) H_2SO_4 at 30°C for 1 hour. The mixture was diluted by adding 7 ml of distilled water, and hydrolyzed at 100°C for 2 hours. The mixture was centrifuged at 10,000 x g for 3 min, and the supernatant was neutralized with 10% (w/v) NaOH. Then, 1 ml of neutralized sample was taken out and added with 1 ml 5% phenol in a centrifuge tube. Mixture was vortexed evenly. The mixture was added with 5 ml concentrated H_2SO_4 and stand at room temperature for 1 hour. Finally the sample was read with spectrophotometer at absorbance of 490 nm.

Biomass saccharification

Enzymatic saccharification experiments of destarched-oil-palm residues were performed in 50 ml schott bottle at 50°C and rotated at 180 rpm for 72 hours. Briefly, oil-palm residues at a 5% (w/v) loading were resuspended in 50 mM sodium acetate buffer solution (pH 5.0). Enzymes preparation of *Penicillium rolfsii* and 2 types of commercial enzyme (Celluclast 1.5L and Accellerase®1500) were adjusted to the same enzyme loading of 6 FPU/g, 10 FPU/g, 14 FPU/g and 20 FPU/g of substrate. Samples were taken from the reaction mixture periodically for sugar analysis by phenol-sulfuric acid method. Total sugar conversion was calculated as follows:

$$\text{Total sugar conversion (\%)} = \frac{\text{Total sugar content obtained after hydrolysis} \times 100}{\text{Total carbohydrate content in the substrate}}$$

Results and Discussions

Chemical composition of destarched-oil-palm (*Elaeis guineensis*) residues mainly derived from inner part of oil-palm trunks was determined as follows: holocellulose (cellulose and hemicellulose), 73.12%; lignin, 24.6%, moisture content, 3.72%; ash, 1.36% and alcohol benzene extractive, 0.91% (Figure 1). These results obtained from Toray Techno Co. Ltd., Japan showing the quite similar compositional content of oil-palm trunk compared to other reports (H`ng *et al*., 2011, Jung *et al*., 2011).

Comparison of *P. rolfsii* crude enzyme and commercial enzymes revealed that *P. rolfsii* crude enzyme showing the superiority of saccharification efficiency for the production of reducing sugars from lignocellulosic biomass (Figure 2). With enzyme loading of 6, 10, 14 and 20 FPU/g substrate of crude enzyme of *P. rolfsii* added in the saccharification system, all different dosage of enzymes showed increment of total sugar conversion sharply within 12 hours and reached the highest levels at 48 – 72 hours. There is no advantage in extending the saccharification when enzyme loading more than 14 FPU/g of crude enzymes added. Total sugar conversion of 14 – 15% was achieved by crude enzyme of *P. rolfsii* on hydrolysis of oil-palm trunk residues.

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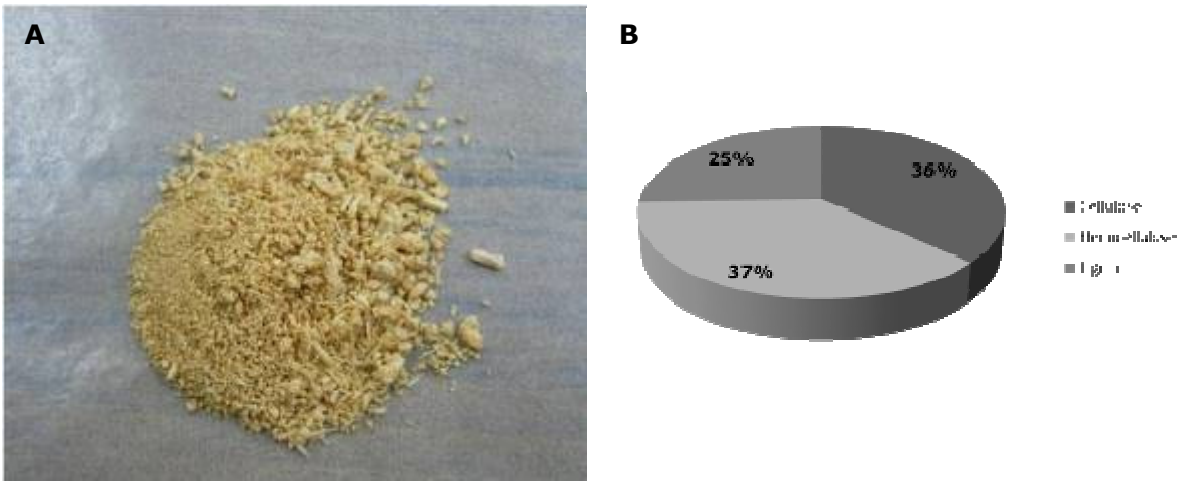

Figure 1. The solid powdery residues which were derived from pulverization of oil-palm trunks (A). Compositional analysis of oil-palm trunk residues revealed that it was mainly consisted of cellulose, 36%; hemicellulose, 37% and lignin, 25%.

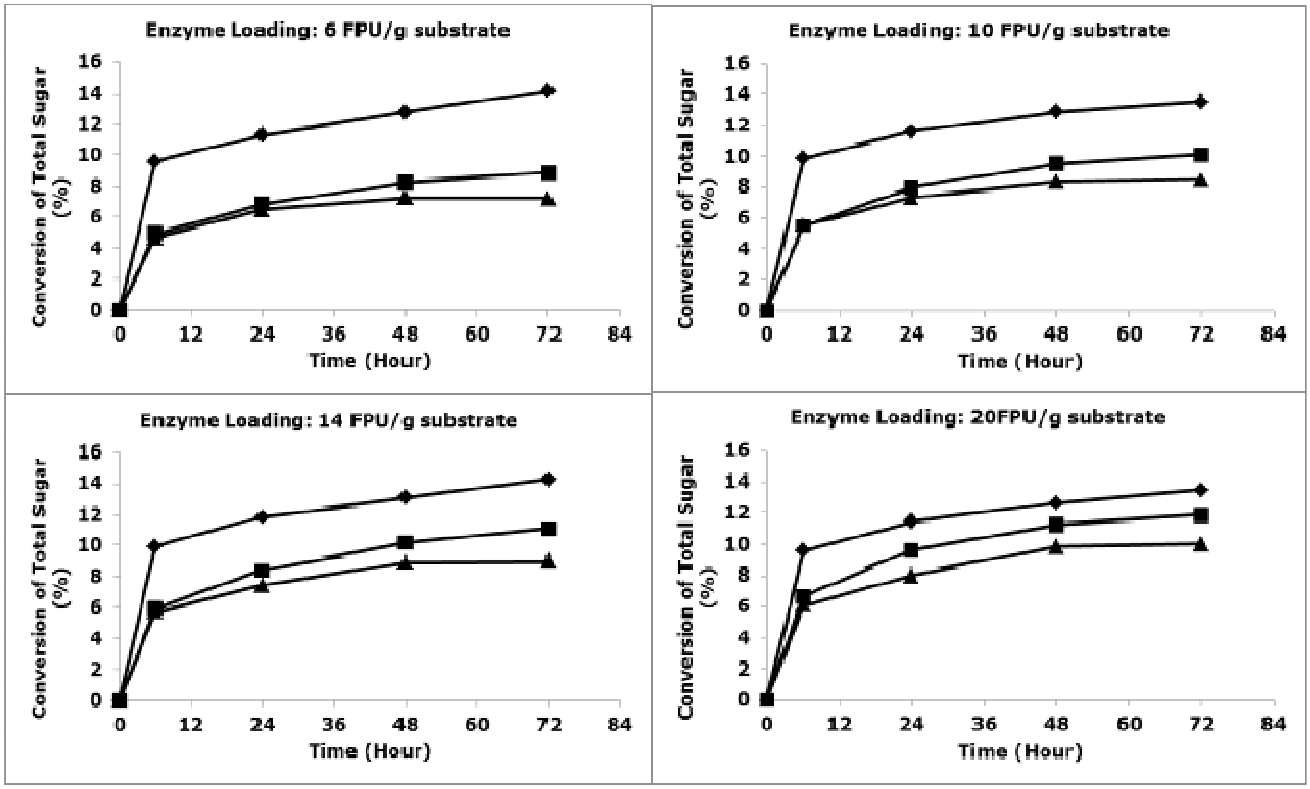

Figure 2. Time-course for hydrolysis of oil-palm residues by using *Penicillium rolfsii* enzyme and commercial enzymes based on hydrolysis of total sugar conversion (%). Data points are the average of duplicate samples. ♦*Penicillium rolfsii*; ■Celluclast 1.5L; ▲Accellerase®1500

Conclusions

Comparison results of crude enzymes with commercial enzymes revealed that *P. rolfsii* was able to produce higher hydrolytic efficiency of enzymes compared to enzyme cocktails of Celluclast 1.5L and Accellerase 1500. Further study need to be conducted such as pretreatment in deconstruction of oil-palm trunk residues for improving the accessibility of enzymes to the substrate so that enhancing the surface area for hydrolysis.

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