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Sugar-Rich Hydrolyzates from Coffee Pulp Waste which Produced under Solid State Fermentation by *Pestalotiosis* sp. VM9 and *Aspergillus* sp. VTM5, and Its Efficiency as Medium for Single Cell Protein *Saccharomyces cerevisiae*

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Sugar-Rich Hydrolyzates from Coffee Pulp Waste which Produced under Solid State Fermentation by *Pestalotiosis* sp. VM9 and *Aspergillus* sp. VTM5, and Its Efficiency as Medium for Single Cell Protein *Saccharomyces cerevisiae*

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Abstract. Polysaccharides rich coffee pulp is one of some abundant agricultural waste in Indonesia. The pulp also contains protein which may lead to be an important source for industrial bioprocessing. Under solid-state fermentation (SSF) by *Pestalotiosis* sp VM9 using of this pulp, reducing sugar was produced 392.35 µg/ml after 6 days incubation at 30ºC. Meanwhile, SSF of the pulp by *Aspergillus* sp. VTM5, after 5 days of incubation 446.6 µg/ml of reducing was released. The investigation proved that the hydrolyzates medium which were produced under SSF by *Pestalotiosis* sp VM9 and *Aspergillus* sp. VTM5 can be used as a source for single cell protein (SCP) *Saccharomyces cerevisiae* production. The SCP productions using both hydrolyzates were$1.89x10^6$ and $2.9x10^6$ cell/mL after 54 and 48 hours of incubation at 30ºC. Furthermore, during SCP production, *S. Cerevisiae* consumed sugars as carbon sources in a range of 189.8- 225.5 µg/ml (49.5-51.6%) from initial concentration. From the results, it is suggested that the coffee pulp waste can be used as a cheap medium for SCP production. Further investigation to improve SCP production efficiency, optimizing of hydrolysis under SSF and analysis of hydrolyzates component were needed.
Keyword: *Aspergillus*sp.VTM5, Coffee pulp, *Pestalotiosis* sp. VM9, SSF, SCP

1. Introduction

During coffee bean processing, a major component as about 45% coffee pulp wastes was generated [1]. A huge amount with approximately 336.6 million tons every year were produced in Indonesia [2][3]. Up today, this waste is not a useful product with less economic value. Coffee pulp still also contained some secondary metabolites such as caffeine, tannin, and polyphenol [4] [5] which cause serious pollution for the environment. In addition, high C/N ratio in coffee reaches to 57.2, made difficult to be decomposed in nature[6]. In contrast, this waste has some potential compounds such as of 63% dry weight of polysaccharides as cellulose, 17% lignin, 2.3% hemicellulose [6] and some nutrient that may lead being potential for industrial bioprocessing, such as briquettes and pelslets [7], bioethanol[8]; [9]; [10]; [11], and biogas [12]. It was also reported through bioconvertion, the coffee pulp waste can be used as raw material for SCP production.

SCP is a cell of microorganism (algae, bacteria, fungi, and yeast), which can be used as a protein ssupplement for human food or animal feed [13]. In SCP production, *S. cerevisiae* has advantages because the larger size of the cell, easier to harvest, lower nucleic acid content, high lysine content, and wide range tolerance of pH compared to other microorganisms [14]. Unfortunately, *S. cerevisiae* cannot synthesize *de novo* simple sugars like algae. In the previous investigation, filamentous fungi

Pestalotipsis sp. VM9 and *Aspergillus* sp VTM5 isolated from decomposed oil palm fruit bunch, produce cellulase and readily hydrolyzed polysaccharide of oil palm fruit bunch substrate into simple sugars under SSF[15].The same manner,these two fungi could utilized coffee pulp as carbon and nitrogen source. And during SSF of coffee pulp by introducing of these two fungi, the sugar rich hydrolyzates were produced which can be used as medium for SCP production. Detail investigation and utilization of its hydrolyzates for medium in SCP production were explained in this paper.

2. Materials and Methods

2.1. Inoculum preparation

Pestalotiosis sp. VM9 and *Aspergillus*sp. VTM5 were pre-cultured on slant Potato Dextrose Agar. These were incubated at 37ºC for 3 days of incubation. The Inoculum size used 10^7 spores/ml from each fungus was 10^7 spores/ml after observed using a Petroff-Hauser counting chamber. SCP *S. cerevisiae* as inoculum was pre-cultured used YPD (Yeast Pepton Dextrose) media and incubated on 125 rpm 30ºC for 3 days. 100 uL of SCP *S. cerevisiae* was added into 50 ml of each hydrolysate. $1.2x10^6$ cells/mL of *S. cerevisiae* were used as initial inoculum size for SCP production.

2.2. Optimization and up-scaling of reducing sugars production on coffee pulp substrate under SSF

Hydrolysis optimization was performed by inoculated 1 mL suspension of spore from both *Pestalotiosis* sp. VM9 and *Aspergillus* sp. VTM5 into 10-gram coffee pulp with water content was 20 mL (1:2) [16]. Then, these were incubated at 37ºC for 1-7 d [17]. Reducing sugar was harvested every day and calculated reducing sugars content using Nelson-Somogy method[18]. Harvesting of reducing sugar was performed by adding 2 ml/gr substrate of aquadest containing 0.01% Na Azide + 1% NaCl or (v/v = solvent/substrate) was 2:1[19]. This mixture was incubated on a shaker for 12 hand 30ºC of temperature. Extraction methods used filtration and centrifugation of mixture with 8000 rpm for 15 min. Up-scaling of reducing sugars production used 500 gram of substrate and incubated at 37ºC for optimum incubation time based on the previous result of hydrolysis optimization experiment.

2.3. Production of SCP S. cerevisiae used fermented coffee pulp

Total 50 ml of sterile coffee pulp hydrolyzate from each fungus was added 100 µL SCP *S. cerevisiae* and incubated on shaker 120 rpm for 1-60 hours. Samples for reducing sugar and cell number measurement were taken in every 6 hof incubation. Reducing sugars and cell number were measurements using Nelson-Somogyi method [18] and spectrophotometric machine with wavelength 600 nm, respectively.

2.4. Reducing sugars assay

Reducing sugar in hydrolyzate was observed using Nelson-Somogy method [18]. Separating cell from the mixture was performed by centrifugation in 10.000 rpm for 10 minutes. 500 µL of hydrolyzate was mixtured by 500 µl Somogyi reagent. Then, it was boiled for 15 minutes, added 500 µl Nelson reagent and 2.5 mL H_2O after the mixture was cold. 1 mL sample was used for absorbance measurement using spectrophotometer with wavelength 500 nm. The absorbance value was converted to glucose concentration based on the standard curve.

3. Results and Discussion

3.1. Optimization of SSF for simple sugars production

Optimization of simple sugars production used filamentous fungi *Pestalotiosis* sp VM9 and *Aspergillus* sp VTM5 on coffee pulp waste under solid state fermentation were known that the optimum reducing sugar produced by *Pestalotiopsis* sp. VM9 and *Aspergillus* sp. VTM5 were 88.3 µg/mL and 28 µg/mL during 6 and 5 days of incubation, respectively (Figure 1). Although, it was not observed yet about the

kind of it reducing sugar. Moreover, the different lignocellulolytic fungi also had different ability to degrade a similar substrate.

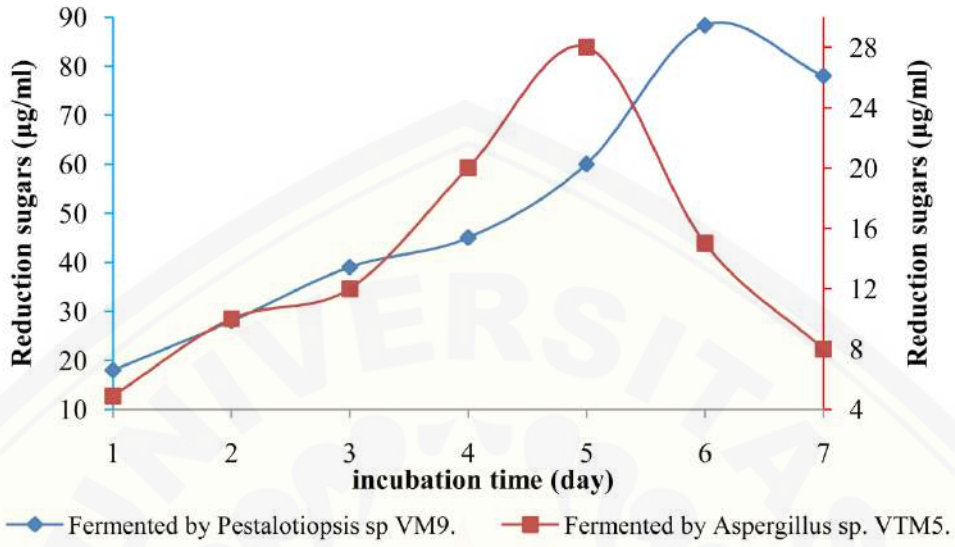

Figure 1. Effect of incubation time for reducing sugars production by *Pestalotiosis* sp. VM9 (blue curve) and *Aspergillus* sp. VTM5 (red curve).

Reducing sugars were produced increasing during the addition of incubation until the optimum time at 6 days using *Pestalotiosis* sp. VM9 and 5 days for *Aspergillus* sp. VTM5. After that, reduction sugars were decreasing. It may be caused some factors such as produced simpler sugars was used first for fermentation microbial growth as carbon source [20]. Moreover, simple sugars were produced under solid state fermentation using each filamentous fungus can to be a feedback mechanism. It means that the final product from a metabolic pathway will be a stopping compound for synthesis pathway itself[21]. The metabolic product such as glucose will be act as inhibitor factor for lignocellulolitic enzyme exspecially cellulase through binding to the allosteric site of the enzyme, so the active site can't make a reaction to the specific substrate [22].

3.2. SCP *S. cerevisiae production using coffee pulp hydrolyzate*

Based on the result of each hydrolyzate fermented by *Aspergillus* sp. VTM5 and *Pestalotiosis* sp. VM9 were known that *Aspergillus* sp. VTM5 had the ability to hydrolize coffsee pulp substrate more effective compared to *Pestalotiosis* sp. VM9. its reducing sugars content fermented by *Aspergillus* sp. VTM5 were relatively higher that another filamentous fungus, namely 446.6 µg/mL and 392.35 µg/mL respectively on each optimum incubation time (Figures 2 and 3).

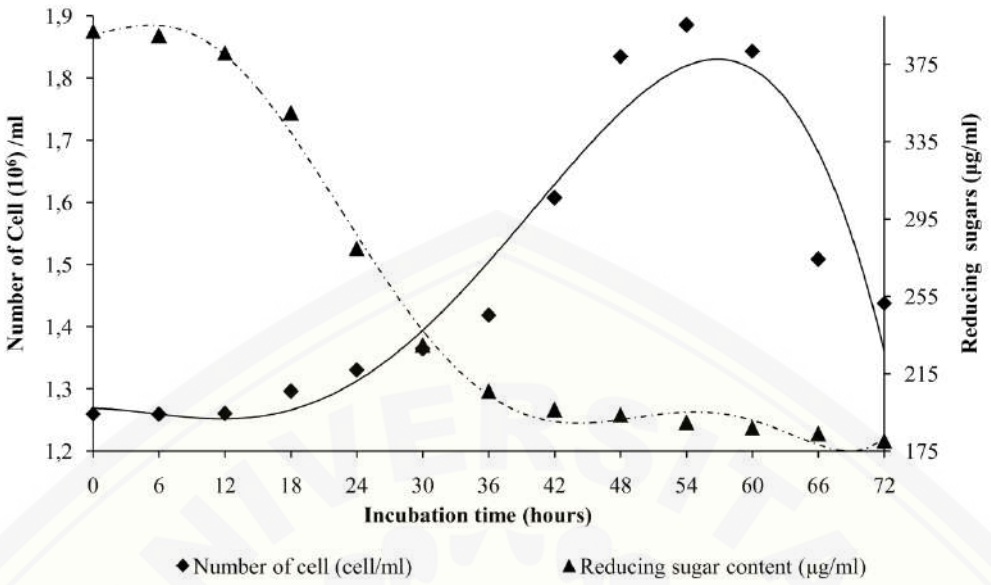

Figure 2. Cell number of SCP *S. cerevisiae* in coffee pulp hydrolyzate fermented by *Pestaslotipsis* sp. VM9.

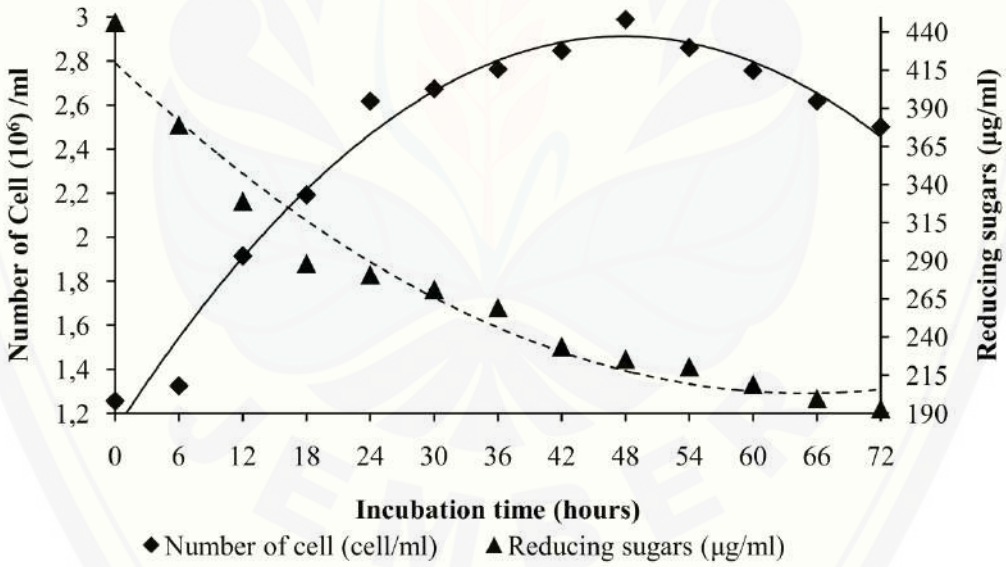

Figure 3. Cell number of SCP *S. cerevisiae* in coffee pulp hydrolyzate fermented by *Aspergillus* sp. VTM5.

Optimum growth of SCP *S. cerevisiae* on each coffee pulp hydrolyzate ware also followed decreasing of reducing sugar (Figures 2 and 3). These reduction sugars more decreased until the last incubation time (72 h), in spite of the growth curve of *S. cerevisiae* have been passed stationary phase or started to the dead phase. This causes these cell of *S. cerevisiae* still need some nutrient to life including to reducing sugars in spite of can grow anymore. So, the reducing sugars were still decreasing.

The result of counting numbers of cell *S. cerevisiae* in every incubation time was known that this species grew better on hydrolyzate fermented by *Aspergillus*sp. VTM5 compared to another hydrolyzate under solid fermentation using *Pestalotiosis* sp. VM9, with the number of cell SCP *S. cerevisiae* reached to 2.99×10^6 cells/mL during 48-54 hours of incubation and 1.89×10^6 cells/mL at 54 h as the optimum

incubation time, respectively (Figures 2 and 3). Total reducing sugar consumption of *S. cerevisiae* was a little bit different between hydrolyzate fermented by *Pestalotiosis* sp. VM9 and *Aspergillus* sp. VTM5 namely 51.6% and 49.5% (Figure 4). However, % growth cell of SCP *S. cerevisiae* on each hydrolyzate fermented by *Pesatalotiopsis* sp. VM9 and *Aspergillus*sp. VTM5 were significantly different, 49.67% and 137.1% respectively (Figure 5). It indicated that coffee pulp waste fermented by *Aspergillus*sp. VTM5 was more effective for SCP *S. cerevisiae* cultivation than solid state fermentation using *Pestalotiosis* sp. VM9. The different growth result of SCP *S. cerevisiae* on coffee pulp hydrolyzate under solid state fermentation using *Aspergillus* sp. VTM5 can be caused kind of the reduction sugar is simpler and can be used as C source directly [23, 24].

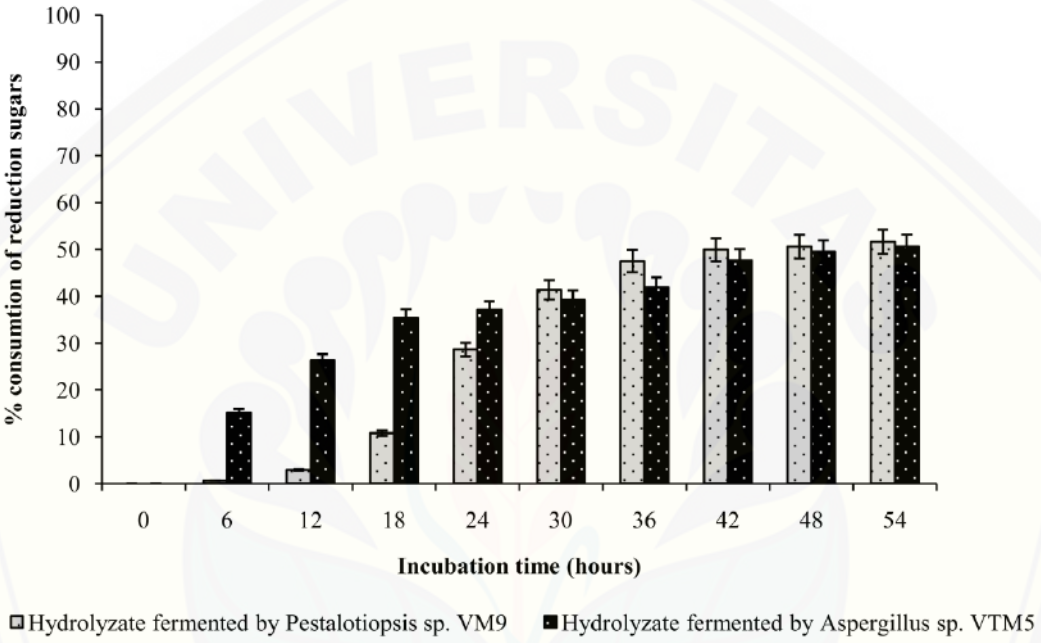

Figure 4. % consumption of reducing sugars by *S. cerevisiae* in each hydrolyzate.

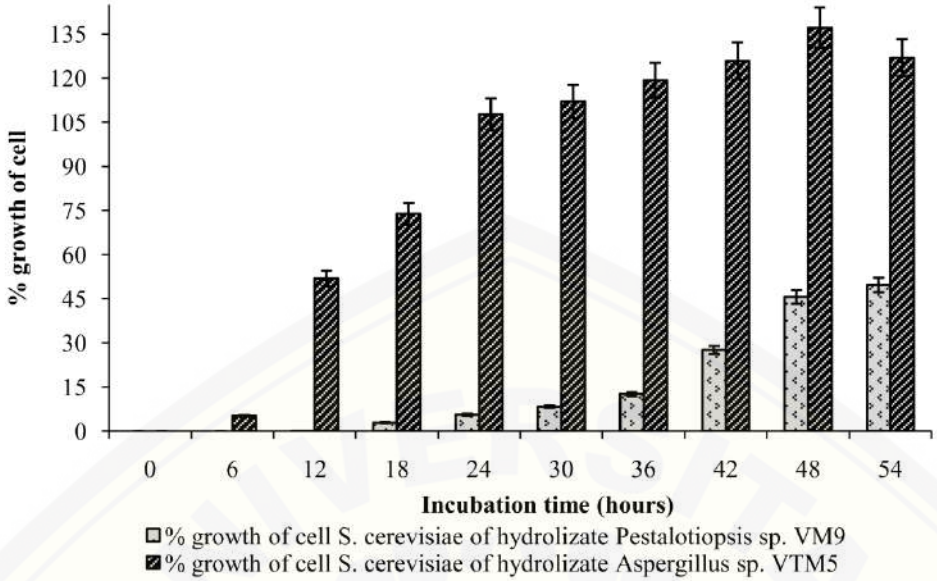

Figure 5. % growth of cell nsumber *S. cerevisiae* in each hydrolyzate.

4. Conclusion

Coffee pulp hydrolyzate fermented by *Aspergillus* sp. VTM5 had higher reducing sugars than another hydrolyzate using *Pestalotiopsis* sp. VM9. It could be a potential cheap substrate for producing SCP *S. cerevisiae* and could be an alternative problem solving for coffee pulp waste management. Although, it still needed further optimizing method

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