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BIODEGRADATION OF PALM OIL EFFLUENT BY CONSORTIUM OF *Bacillus* sp., *Phanerochaete chrysosporium* and *Trichoderma viride*

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Abstract

Manufacture of crude palm oil (CPO) is one of Indonesia's industrial agro commodities that support the Indonesian economy. The increase in CPO production will increase the production of waste palm oil (Elaeis guinensis Jacq.). Palm oil mill wastewater has high potential to pollute the waters. This is due to palm oil wastewater containing high levels of COD (Chemical Oxygen Demand), and BOD (Biochemical Oxygen Demand), TSS (total suspended solid), containing solids of crude Palm oil, smelling, dark brown color, contain high levels of oils, fats and proteins as well as the rest of the cooking pressed palm fruit bunches. The purpose of this study was to determine the ability of the consortium of Trichoderma viride, Phanerochaete chrysosporium and Bacillus sp. and effective dose inoculums in degrading oil palm wastewater. This study used an experimental method to completely randomized design (CRD) factorial with two factors and three replications. Factor 1 consortium type are c1 (Bacillus sp.,Trichoderma viride), a consortium of c2 (Bacillus sp.,P hanerochaete chrysosporium) and a consortium c3 (Bacillus sp., Phanerochaete chrysosporium and Trichoderma viride) and factor 2 inoculums dose are; 0% (no inoculation), 2.5% and 5%. The results showed that the consortium of Bacillus sp., Phanerochaete chrysosporium and Trichoderma viride at a dose of inoculum 2.5% most effective in degrading wastewater palm oil, because it produces a reducing sugar levels 64.27%, lower levels of BOD by 44%, reduce levels of COD by 25.86%, reduce levels of ammonia by 60.71% and lower levels of TSS by 46.43% within 9 days.

Keywords*: Biodegradation, BOD, COD, Trichoderma viride, Wastewater Palm Oil.*

INTRODUCTION

Palm oil industry is one of the agro strategic industries. Some countries in tropical regions such as Indonesia, Malaysia, and Thailand are a major supplier of World CPO (Crude Palm Oil). Fresh fruit bunches (FFB) palm oil, which is produced from palm oil plantations, processed into crude palm oil (CPO). CPO is important for agro industrial products to be processed into derivatives oil food and non-food industry. Indonesia palm oil plantation is about 13.5 million hectares (Info Sawit, 2013).
Palm oil production is expected to reach 28 million tons, with compositions ranging from 17-18 million tons were exported mainly to India, China, and Europe, the Indonesian government further ensure the palm oil industry to supply the raw material in the production of biodiesel and diesel fuel is now producing 2.3 million tonnes per year (Info Sawit, 2013). The increase in palm oil production will have an impact on increasing the amount of waste generated. Wastewater from palm oil processing has the potential to pollute the waters because of high organic matter content and low acidity, so it needs to be in remediation before being discharged into water bodies. In Indonesia, palm oil mill effluent (MCC) is estimated to produce 28.7 million tons of waste/year (Mahajoeno, 2010). In general, the characteristics of palm oil wastewater containing high organic material, suspended material and fat, so have high levels of COD (Chemical Oxygen Demand) BOD (Biochemical Oxygen Demand), solids crude palm oil, protein and fibre as well as the rest of the stew-pressed fruit palm oil (Naibaho, 1996). The main components of the oil palm waste are cellulose, lignin and lipids (triglycerides) that are known as lignocelluloses cellulose content reached 54% (Darnoko, 1993). If the waste is directly discharged into the river most of it will settle, decompose slowly, consume dissolved oxygen, causing turbidity, produce a very pungent odour, and damage the fish breeding areas.

Therefore, it is necessary wastewater biodegradation of oil palm to reduce pollution levels before discharge to the environment. Lignocellulolytic and lipolytic microorganisms to decompose cellulose components, and lipids in lignocelluloses wastes conduct palm oil wastewater biodegradation process.

Biodegradation of these components is done by the genus Bacillus and *Trichoderma viride* that produce relatively large amounts of cellulose enzymes. *Bacillus* sp. also degrade cellulose polymer other compound such as lipids, proteins, starch and pectin. *Trichoderma viride* is capable to break down the cellulose complex. While the fungus has the ability to degrade lignin is *Phanerochaete chrysosporium*. In addition, having the ability to degrade lignin, the fungus can also degrade cellulose and hemicellulose (Wymelenberg, 2006).

According to Wizna et al. (2005) inoculum starter concentration as much as 6% of *Bacillus* sp. can degrade cellulose fibre content in fermented cassava waste containing high cellulose by 32% for 6 days. Meanwhile, according to research conducted Leviolifia (2010) inoculums concentration of 5% from *Bacillus* sp. can reduce cellulose content of 34.48 %. *Phanerochaete chrysosporium* is a white rot fungi degrade lignin is known for his ability and cellulose (Cookson, 1995). *Phanerochaete chrysosporium* can degrade cellulose to speed up the composting process slugde waste paper industry; the inoculums concentration of 5% can reduce the content of cellulose in the paper industry wastewater by 24.51%. According to research conducted Christyanto and Subrata (2005), *Trichoderma viride* can reduce levels of cellulose in bagasse 50.13%. Wastewater palm oil used in this study is wastewater from oil palm aerobic ponds of palm oil mill PT. Sawita Inti Perkasa, Medan, North Sumatra with the characteristics of the wastewater is brown, slightly viscous and odourless. Early test had higher levels of BOD of 6390 mg/l, COD content of 18.285 mg/l, TSS levels of 17025 mg/l and a pH value of 4.14. The purpose of this study was to determine the ability of the consortium of *Bacillus* sp., *Phanerochaete chrysosporium* and *Trichoderma viride* in wastewater biodegradation of oil palm. The goal is to obtain a microbial consortium and inoculums doses that effective to be applied for palm oil wastewater.

MATERIALS AND METHODS

Culture of *Bacillus* sp., *Trichoderma viride Phanerochaete chrysosporium*, medium starter for fungal include: rice flour, corn cob, corn starch molasses, Palm oil effluent from pond, Reagent for DNS (dinitrosalisilat acid) test, reagents for the determination of BOD, COD, reagent for determination of ammonia: Nessler and Siegnet solution. The method used in this study is an experimental method using a completely randomized design (CRD) 3x3 factorial with three replications. The first factor is the consortium of *Bacillus* sp., *Trichoderma viride* (c1), *Bacillus* sp., *Phanerochaete chrysosporium* (c2), *Bacillus* sp., *Phanerochaete chrysosporium, Trichoderma viride* (c3). Factor II is a starter Inoculum cons. (D), 0% (d0), 2.5% (d1), 5% (d2). The study was conducted for 9 days. Parameters in this study were the levels of BOD (Biochemical Oxygen Demand) by iodometric titration method (APHA, 1985), the levels of COD (Chemical Oxygen Demand) using iodometric titration method, the levels of TSS (Total Suspended Solid) with the gravimetric method using Whatman filter paper 0.45 μm (Ministry of Public Works, 2007), Determination of ammonia levels by using the spectrophotometric method (APHA, 1989). Determination of Reducing sugar DNS. Data were analyzed using analysis of variance (ANOVA). If the results are significantly different then continue with Multiple Range Test (Duncan) with a 5% significance level.

RESULTS AND DISCUSSIONS

Wastewater biodegradation by consortium to reducing sugar content in the inoculums doses of 2.5% and 5%.

Cellulose is the main component on palm wastewater, trough the hydrolysis process the cellulose were converted into more simple sugar or reducing sugar. Cellulose is composed of 15,000 D-glucose residues (Voet and Voet, 2004). Sub unit of glucose join together because there are-1.4 bonds forming the cellulose. Reducing sugar is a sugar group, which can reduce compounds accepted electron, for example glucose. In process of cellulose biodegradation, glucose is the result of

metabolism microbial cellulolytic because of that, the higher enzymatic hydrolysis ability, the lower cellulose in total. Based on measurement of reducing sugar content for 9 days shows that consortium of *Bacillus* sp., *Phanerochaete chrysosporium* and *Trichoderma viride* with inoculum concentration 2.5% resulted the highest reducing sugar content that is (d2) 26.66% comparing to other consortium. If the consortium compared with treatment no inoculation, reducing sugar levels content, increase above an average 50%. The highest increasing of reducing sugar content is also attained by *Bacillus* sp., *Phanerochaete chrysosporium* and *Trichoderma viride* with each concentration in 2.5% and 5% that is 63.74% and 64.227% which means the microorganism species produce cellulose enzyme that hydrolyze cellulose in palm oil wastewater.

Table 1. Percentage reducing sugar content (%) in biodegradation palm oil wastewater process

	Concentration of inoculums 2.5%			Concentration of inoculums 5%			0%
	Bacillus sp. & *T. viride*	*Bacillus* sp. & *P. chrysosporium*	*Bacillus* sp. & P. chrysosporium & *T.viride*	*Bacillus* sp. & *T. viride*	*Bacillus* sp. & *P. chrysosporium*	*Bacillus* sp. & *P. chrysosporium* & *T. viride*	
H0	63.54	57.95	56.52	60.18	74.61	68.88	27.95
H9	70.77	57.58	77.07	67.41	67.41	78.23	22.88
(%) reduction sugar level during 9 days	10.22	0.64	**26.66**	10.73	10.68	11.95	22.16
(%) reduction sugar level compare with Control	60.51	51.46	63.73	58.54	58.54	**64.27**	22.16

Trichoderma viride can produce much cellulose enzyme and hemicelluloses on commercial scale and already being used on pulp, paper, and herd food and textile industry. This enzyme is also being used to sacarification plant biomass into simple sugar on bioethanol production (Seiboth et al., 2011).

Bacillus could produce much hydrolyze polysaccharide extracellular enzyme even though carbocsimetillulase cannot hydrolyze crystalline cellulose. But, study in endogluconase, bacillus and fungi shows activity on microcrystalline cellulose (Yin et al., 2010). Based on cellulose degradation mechanism, cellulose divided into non-processed cellulose (endocellulose) or processed cellulose (including different of exocellulose). Endocellulose randomly cut cellulose into open position and produces new reduction point. But, processed cellulose still attached on chain and releases units especially cellobiose or cellotetraose from one of the point. While cellulose degrades, non-processed cellulose and processed cellulose work on synergize. Generally, effective biological cellulose need synergize action from 3 enzyme that is endo-β-1.4-glucanase (EC.3.2.1.4), cellobiohydrolasey (EC.3.2.1.91), and β-*D* -glucosidase (EC.3.2.1.21). The enzymeendo- ß-1.4 glucanase have systemically name ß-1.4-D-Glucano hydrolase (EC. 3.2.1.4). This enzyme hydrolyze glicosidic bond ß-1.4 randomly and work especially in amorf area from cellulose fiber, like Carboxy Methyl Cellulose (CMC). Enzyme ß-1.4-D-Glucan cellobiohydrolase (EC.3.2.1.91), attack chain point Cellulose non reduction and produce cellobiose and ß-1.4-Glucoxidise has systemically name ß-1.4- Glucoxide Gluco hydrolase (EC.3.2.1.21), hydrolyze EC 3.2.1.21 cellobiose and short chain cello-oligosaccharidez and produce D Glucose (Yin et al., 2010). Now, cellulose generally used in many industries such as animal feed, textile, wastewater and winery. Lignocelluloses enzyme produced by *Phanerochaete chrysoporium* has ability to broke lignin with delignification process then hydrolyze cellulose into simple glucose that can be used as protein source as known that empty palm cluster contain high fiber. The main content of palm cluster is cellulose and lignin. Cellulose enzyme produced by *Bacillus* sp., *Phanerochaete chrysosporium* and *Trichoderma viride* is an enzyme that can break down cellulose palm oil wastewater into simple glucose molecules that more simple decreased levels of cellulose content could happen when high cellulosic activity to degrade and hydrolyze cellulose into

more simple glucose. So the bacteria that inoculated can use those compounds as nutrition source in biodegradation process.

Wastewater biodegradation by the consortium against BOD (Biochemical oxygen demand) content on inoculums doses of 2.5% and 5%.

Biochemical oxygen demand is amount of oxygen needed by microorganism under water to divide or degrade or oxidize organic waste under the water. 9 days during palm oil wastewater degradation process shows that consortium c1; *Bacillus* sp. and *Trichoderma viride* decrease BOD content with 2.5% inoculums concentration as 34.76%. In 5% concentration of *Trichoderma viride* and *Bacillus sp*, consortium only shows decreasing BOD content by 9.29%. BOD content on palm oil wastewater on every consortium treatment is lower than controlled palm oil wastewater. The highest decrease compare to control (d0) achieve by consortium *Bacillus* sp. *Phanerochaete chrysosporium* and *Trichoderma viride* in 2.5% (d1) and 5% (d2) inoculums concentration, each of them is 44% and 40%.

Trichodermaviride produce cellulolytic enzyme that is able to decompose cellulose in the palm of wastewater result in lower levels of BOD (biochemical oxygen demand). *Phanerochaete chrysophorium* produce lignolytic enzyme, which consist of peroxide lignin, peroxide manganese and laccase that could degrade organic compound on lignin in palm oil wastewater. *Bacillus* sp., *Phanerochaete chrysosporium* and *Trichoderma viride* consortium ability decrease the highest BOD content can be happen because the three species produce enzymes that synergize to break down the main component of palm oil waste were cellulose, lignin and lipid. According to Roheim (2011), Bacillus sp could degrade lipid into glycerol and fat acid so it would be easier digested by microbe as one of nutrition source. *Trichoderma viride* produce cellulolytic enzyme that is able to decompose cellulose in the palm of wastewater resulting in lower levels of BOD (biochemical oxygen demand). *Phanerochaete chrysophorium* produce lignolytic enzyme, which consist of peroxide lignin, peroxide manganese and laccase that could degrade organic compound on lignin in palm oil wastewater. According to Saritha et.al (2010), the decreasing BOD process, the main quality of palm oil wastewater is 100 mg/l, but a good decreasing percentage shows that species consortium which been used succe-ssfully decreasing BOD content. Efficiency percentages of content decreasing BOD that not maximal thought to be caused by the BOD content were really high or really dense so it takes more time. The thickness of palm oil waste causes the low oxygen supply, which come and dissolved. Increasing in oxygen content would lower BOD and COD content.

Wastewater biodegradation by the consortium against COD (Chemical oxygen demand) content on inoculums doses of 2.5% and 5%.

Chemical Oxygen Demand is oxygen amount needed to analyze every organic material that contained in water (Boyd, 1990).

Table 2. Decreasing percentage of BOD (mg/l) in biodegradation palm oil wastewater process

	Concentration of Inoculum 2.5%			Concentration of Inoculum2 5%			0%
	Bacillus sp. & *T. viride*	*Bacillus* sp. & *P. chrysosporium*	*Bacillus* sp. & *P. chrysosporium* & *T. viride*	*Bacillus* sp. & *T. viride*	*Bacillus* sp. & *P. chrysosporium*	*Bacillus* sp. & *P. chrysosporium* & *T. viride*	
H0	4.634	3.658	2.439	3.252	3.089	2.682	4.796
H9	3.023	3.050	2.700	2.950	2.950	2.875	4.308
(%) reduction level of BOD during 9 days	34.76	16.62	10.70	9.29	4.50	**0.20**	10.18
% reduction level of BOD compare to Control	37	36	44	38	38	**40**	10.18

Table 3. Decreasing percentage of COD (mg/l) in biodegradation palm oil wastewater

	Concentration of Inoculum 2.5%			Concentration of Inoculum2 5%			0%
	Bacillus sp. & *T. viride*	*Bacillus* sp. & *P. chrysosporium*	*Bacillus* sp. & *P. chrysosporium* & *T. viride*	*Bacillus* sp. & *T. viride*	*Bacillus* sp. & *P. chrysosporium*	*Bacillus* sp. & *P. chrysosporium* & *T. viride*	
H0	66.334	64.740	63.903.2	60.756	65.736	73.708	77.688
H9	67.300	57.300	57.600	66.800	72.800	52.800	68.800
(%) reduction level of COD during 9 days	1.46	11.49	9.86	9.95	10.75	**28.37**	11.44
% reduction level of COD compare to Control	13.37	26.24	25.86	14.02	6.29	**32.04**	11.44

Phanerochaete chrysosporium and *Trichoderma viride* able to decrease COD content until 32.04% with 5% inoculums concentrate (D2). The ability to reduce levels of COD due to palm oil waste Bacillus sp. can degrade lipids in wastewater into glycerol and fatty acids, enzymes produced by *Trichoderma viride* cellulase breaks down cellulose into glucose (Wang, 2010).

Phanerochaete chrysosporium produces lignin peroxides enzyme lignolytic form, which is able to degrade lignin complex organic compounds into simple sugars. COD levels of biodegradation by the consortium still exceed the maximum levels of COD in the effluent quality standard palm oil is equal to 350 mg/l. This is because of the levels of COD in the effluent was very high, and the process of biodegradation is only done for 9 days.

Wastewater biodegradation by the consortium against ammonia content on inoculums Doses of 2.5% and 5%.

Ammonia is a compound derived from a variety of pollutant compounds, namely NH-3-N or NO 2-N. Ammonia in waters generated by the decomposition process, the reduction of nitrate by bacteria, fertilization and excretion of the organism that are inside (Boyd, 1990). Any ammonia that is released into the environment will form an equilibrium reaction with ammonium ions ($NH4^{+}$). Ammonium is a nitrogen compound that is toxic at high levels, which can degrade the quality of water, which is the source of life for living organisms, especially aquatic biota.

Table 4. Decreasing percentage of ammonia content (mg/l) in biodegradation palm oil wastewater process

	Concentration of inoculums 2.5%			Concentration of inoculums 5%			0%
	Bacillus sp. & *T. viride*	*Bacillus* sp. & *P. Chrysosporium*	*Bacillus* sp. & *P.chrysosporium* & *T. viride*	*Bacillus* sp. & *T. viride*	*Bacillus* sp. & *P. chrysosporium*	*Bacillus* sp. & *P. chrysosporium* & *T. viride*	
H0	0.15	0.14	0.19	0.17	0.11	0.14	0.28
H9	0.1	0.07	0.15	0.12	0.1	0.12	0.11
(%) Reduction level of Amoniakduring 9 days	33.33	**50**	21.05	29.41	9.09	14.29	60.71
% Reduction level of Amoniak compare to Control	64.29	**75.00**	46.43	57.14	60.71	57.14	60.71

The genus Bacillus can also reduce levels of ammonia, due to its ability to oxidize ammonia content of the waste (Edwards, 2011). Consortium of *Bacillus* sp. and *Phanerochaete chrysosporium* can reduce levels of ammonia with inoculums concentration of 2.5% is equal to 50%. Levels of ammonia in the effluent oil palm inoculated by the consortium are lower than the effluent ammonia levels control oil. Consortium of Bacillus sp. and *Phanerochaete chrysosporium* inoculums at a concentration of 2.5% were able to reduce levels of ammonia amounted to 75.5%.

Biodegradation of waste palm oil consortium *Bacillus* sp., and *Phanerochaete chrysosporium* is the highest in the lower levels of COD. The genus Bacillus can also reduce levels of ammonia, due to its ability to oxidize ammonia content of the waste (Edwards, 2011). An enzyme produced by *Phanerochaete chrysosporium* lignocelluloses is a simple break down lignin into glucose, which is used as a

source of nutrition for nitrifying bacteria. Quite a high percentage of decrease in ammonia levels in the effluent oil palm is also suspected due to the change of ammonia into a gas that is released into the air through evaporation process (volatilities).

According Irianto (2007), the levels of nitrogen in the form of ammonia, nitrate and nitrite can be reduced through amonification and nitrification and volatilization. Microbes can be used to enhance the functional activity of the mineralization in aquatic systems. Hydrolysis of peptide compounds by microbial enzymes extra–cellular is the key of mobilization of ammonia and nitrogen cycle in ecosystems (Bach et al., 2001). Nitrifying bacteria have important functions in the nitrogen cycle, which involves the conversion of ammonia to nitrite and then the conversion of nitrite to nitrate. During the process of nitrification, nitrifying bacteria detoxify ammonia in two stag. First, *Nitrosomonas* sp. converts toxic ammonia into nitrite, which is also toxic to fish. The second step, nitrite is converted to nitrate by *Nitrobacter* spp., which is not considered toxic unless accumulate in high concentrations of heterotrophic bacteria such as Bacillus spores that form can replace the role of nitrifying bacteria because it is more tolerant to the environment. Many species of the genus Bacillus produce extra and intra-cellular proteases in addition to amylase, Bacillus is also used as a fertilizer and nitrifier for aquaculture systems and capable to decompose protein complexes, and organic matter into ammonia, subsequent to nitrite and finally into nitrate (Seri Intan, 2005).

Wastewater biodegradation by the consortium against (TSS) Total Suspended Solid content on inoculums Doses of 2.5% and 5%.

Total suspended solids or suspended solids are solids that cause turbidity of the water, not soluble and cannot be settled immediately. Suspended solids composed of particles smaller size and severity of sediment such as certain organic materials, clay and others.

Table 5. Decreasing percentage of TSS (Total suspended solid; ml/l) in biodegradation palm oil wastewater

	Concentration of inoculums 2.5%			Concentration of inoculums 5%			0%
	Bacillus sp. & *T. viride*	*Bacillus* sp. *P. chrysosporium*	*Bacillus* sp. *P. chrysosporium* & *T. viride*	*Bacillus* sp. & *T. viride*	*Bacillus* sp, *P. chrysosporium*	*Bacillus* sp. *P. chrysosporium* & *T. viride*	
H0	32.000	36.000	33.600	47.600	34.800	38.200	47.970
H9	28.200	24.600	23.600	30.800	12.400	30.400	32.500
(%) Reduction level of TSS during 9 days	13.23	**31.67**	29.76	35.29	64.37	20.42	32.24
% Reduction level of TSS compare to Control	41.21	**48.71**	50.80	35.79	74.15	35.99	60.7

Biodegradation TSS for 9 days showed that the consortium of *Bacillus* sp. and *Phanerochaete chrysosporium* inoculums at a concentration of 5% (d2) is able to reduce the amount of the highest levels of TSS 64.37%. Decreased levels of palm oil effluent TSS at all consortiums are lower than the levels of palm oil effluent TSS control. Consortium of *Bacillus* sp. and *Phanerochaete chrysosporium* with inoculums concentration of 5% (d2) is able to reduce levels of the highest TSS was 74.15%. Consortium of *Bacillus* sp., *Phanerochaete chrysosporium* and *Trichoderma viride* concentrations of 2.5% were able to reduce levels of TSS 50, 80%. While the highest decreasing efficiency is biodegradation by the consortium of Bacillus sp. and *Phanerochaete chrysosporium* with concentration of 5% is at 65.53%. Capability consortium of *Bacillus* sp. and *Phanerochaet echrysosporium* highest in the lower levels of TSS due to the species of microbes produce enzymes that can break down the organic matter content of palm oil wastewater as Bacillus sp. can decompose cellulose fibers and lipids are dissolved in the palm oil wastewater. *Phanerochaete chrysosporium* produces lignin peroxidise can decipher crude fibre and lignin are difficult to decompose through a process of delignification and cellulose hydrolysis so solid that dissolved

organic matter in the wastewater in the form of lignin, cellulose and lipids can be reduced. The number of bacteria and fungi are closely related to decreased levels of BOD, COD and TSS. High microbial populations and activities in line with the amount of enzymes produced.

CONCLUSIONS

The effective consortium on palm oil biodegradation process are *Bacillus* sp., *Phanerochaete chrysosporium* and *Trichoderma viride* consortium can produced reducing sugar content that could reduce BOD, COD, ammonia and TSS content.

Inoculums concentration on palm oil wastewater biodegradation process in 2.5% concentration effective to produce reducing sugar by 63.73% which reduce BOD content by 44%, COD content by 25.86%, ammonia content by 60.71% and TSS score by 46.43% on palm oil wastewater biodegradation process.

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