Aerobic treatment of POME with indigeneous individual and consortium bacteria

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Aerobic treatment of POME with indigeneous individual and consortium bacteria

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Abstract. Palm Oil Mill Effluent contains high value of COD, BOD and TSS being able to pollute the water body. Application of aerobic indigeneous bacteria for treatment of the POME was conducted to reduce the value of COD, BOD and TSS. Bacillus cereus ATCC 14579 (KP 1.1), Pseudomonas azotoformans strain NBRC 12693 (KP 1.3) and Burkholderia cepacia ATCC 25416 (KP 2.2) were used to degrade the components of cellulose, protein and lipase in the POME, respectively. The consortium of bacteria were also applied for degradation of POME. The research was conducted in four bioreactors of 12 litres with variation of time 3, 6, 9, 12, 15, 18, 21 and 24 hours. Parameters observed in the research consisted of bacterial population, COD, BOD, TSS and pH. The experimental results showed that the highest population of bacteria of Bacillus cereus ATCC 14579 (KP 1.1), Pseudomonas azotoformans strain NBRC 12693 (KP 1.3), Burkholderia cepacia ATCC 25416 (KP 2.2) and the bacterial consortium were 8.4 x 107 CFU/ml, 8.5 x 107 CFU/ml, 8.2 x 107 CFU/ml and 9.4 x 107 CFU/ml, respectively. The lowest COD value obtained for those bacteria were 22.6 mg/l, 12.3 mg/l, 14.4 mg/l and 11.8 mg/l, respectively. The lowest BOD values were 9.2 mg/l, 4.4 mg/l, 5.2 mg/l, dan 2.9 mg/l while those of TSS value were 3.2 mg/l, 3.0 mg/l, 4.0 mg/l and 4.2 mg/l. Values of pH ranged from 6.7 to 7.6 for Bacillus cereus ATCC 14579 (KP 1.1), from 6.8 to 7.4 for Pseudomonas azotoformans strain NBRC 12693 (KP 1.3), from 6.9 to 7.5 for Burkholderia cepacia ATCC 25416 (KP 2.2) and from 6.7 to 7.5 for the bacterial consortium.

1. Introduction

Crude Palm Oil (CPO) has been used for production of oleo chemicals, frying oil, cosmetics and biodiesel. CPO has been produced widely and it is consumed all over the world. Indonesia and Malaysia are the most producer of CPO in the world since these countries produces around 85-90% of total CPO production. Increasing consumption of CPO reflects to increase of the palm plantation. In 2017, Indonesia possed 11.9 million hectares in which it achieves three folds of the plantation area in 2000. It is predicted the palm plantation area reaches 13 million hectares in 2020 [1]. Production of CPO in Indonesia around 23 million tons of 640 palm oil mills which is around 46 % of total CPO production in the world. Utilization of biofuel is supported by government of most countries [2]

Palm Oil Mill Effluent (POME) has been produced from sterilization, pressing, decantation units. POME contains dissolved substances including cellulose, hemicellulose, protein, oil and fat, organic compounds and mineral mixture. POME must be treated in the proper way before it is discharged to the environment. The effluent must meet the standard determined by Environmental Ministry Act No. 51 year 1995. Volume of POME ranges from 1 to 1.3 m^3/tons FFB or from 2 to 3 tons of wastewater /tons of palm oil. POME contains of BOD from 20,000 to 30,000 mg/l, COD from 40,000 to 60,000 mg/l [3]. POME consists of water of 95 %, dissolved and suspended solid of 4.5 % and oil and fat of

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0.5 to 1 %. The untreated POME will severe the environment (land or water) due to high content of organic and inorganic compound dissolved in the liquid waste. POME has been treated by using the physical and chemical methods, however these methods have drawbacks due to its expensive treatment. The advantage of biological process in treatment of POME is utilization of bacterial agents continuously. The indigeneous bacteria can be cultivated and used for the treatment of POME.

The biological process is more economical compared than physical and chemical treatment as it does not require high investment cost. The undetermined bacteria utilized in the treatment of POME has weakness in term of the unknown growth rate of the bacteria and synergism of the bacteria is not known.

Research on bacterial isolation from several industrial waste had been carried out. The bacterial isolation was utilized as the bacterial agent for the industrial waste treatment. Kinetic parameters were observed in the process treatment of the wastes. The kinetic parameter obtained for μ_m = 1.128 min^{-1} and Ks = 0.017 mg/l [4]. The value μ_m = 1.240 day^{-1} and Ks = 3.68 g/l [5]. The bacterial isolation was developed and it was tested to achieve the kinetic parameters of μ_m = 0.102 day^{-1}, K_s = 0.555 g/l, k_d = 1.37 x 10^{-16} $hour^{-1}$, the highest Y = 0.4583 mg MLVSS/mg COD [6]. Indigenous bacteria isolated from the POME has been coded as KP 1.1, KP 1.3 and KP 2.2. Oxygen test has been applied for the bacteria and it is categorized as aerobic bacteria. Organic substrate in the wastewater is consumed by the bacteria for its maximum growth [7]. Metabolism activity and growth of organism are affected by temperature [8]. The advantages of aerobic process in degradation of substrate are more ATP produced, more completion of oxidation process and more energy produced. The synergism of indigeneous bacteria of KP 1.1, KP 1.3 and KP 2.2 has been studied. The aims of research are to degrade POME using the indigenous bacteria *Bacillus cereus* ATCC 14579 (KP 1.1), *Pseudomonas azotoformans strain NBRC 12693* (KP 1.3) and *Burkholderia cepacia* ATCC 25416 (KP 2.2) and the consortium, to investigate the bacterial growth, reduction of COD, BOD and TSS and to determine the microbial kinetic parameters in terms μ_{max}, K_s, Y, q_{max} and K_d.

2. Materials and Methods

2.1 Preparation of Mineral Medium

Mineral medium of 2 litres was prepared by mixing 9 gr of K_2HPO_4; 2 gr of $(NH_4)_2SO_4$; 0.4 gr of $MgSO_4.7H_2O$; 0.2 gr of NaCl; 0.2 gr of $CaCl_2$; 0.04 gr of $FeCl_3$; 20 gr of glucose; 4 gr of yeast; 2.000 ml of aquadest. The mixture was heated until all of the materials were dissolved, then it was sterilized in autoclave for 1 hour at 121°C and 15 psi.

2.2 Rejuvenation of Bacteria

Agar nutrient of 20 grams was dissolved in 100 ml of aquadest. The solution was heated evenly and it was poured into 12 reaction tubes in which each of the reaction tube was filled with 7 ml of the solution, then it was strelized for 1 hour in the autoclave. The reaction tube was placed in the slanted position for 2 hours so that its medium solidified in the tube. Each bacteria of KP 1.1, KP 1.3 and KP 2.2 from petridisks was inoculated in four tubes aseptically by zigzag method using ose needle. It was incubated for two days to obtain stock cultures.

2.3 Preparation of Inoculum and Starter of Bacterial Culture

Nutrient broth of 16 grams was dissolved in 2 litres of aquadest to prepare the medium of nutrient borth. The medium was heated and it was poured into three erlenmeyer of 667 ml volume. The medium in the Erlenmeyer was strelized for 1 hour in the autoclave and it is cooled down. The medium was inoculated by culture *Bacillus cereus* ATCC 14579 (KP 1.1), *Pseudomonas azotoformans strain NBRC 12693* (KP 1.3) and *Burkholderia cepacia* ATCC 25416 (KP 2.2) in each medium, then the Erlenmeyer was placed in the shaker for 24 hours.

The starter was prepared in bioreactor of 2 liters filled with each bacterial inoculum that consisted of 500 ml inoculum, 400 ml liquid waste and 1,100 mineral medium. Inoculum of bacterial consortium

was prepared by 167 KP 1.1, 167 ml of KP 1.3 and 167 ml of KP 2.2. The starter of 167 ml required 133 ml of liquid waste and 367 ml of mineral medium. These mixture was poured into erlenmeyer and shaked in the shaker with the speed of 120 rpm at room temperature for 3 hours. Number of cells was observed after 3 hours with direct method until bacterial population achieved 10^6 cfu/ml

2.4 Degradation of Substrate Process

Degradation process of POME was conducted in four separated bioreactors of 12 litre. consisted of POME and bacterial starter. Each of bioreactor was filled with 6 litres of liquid waste, starter of *Bacillus cereus* ATCC 14579 (KP 1.1), *Pseudomonas azotoformans strain NBRC 12693* (KP 1.3) and *Burkholderia cepacia* ATCC 25416 (KP 2.2) and the bacterial consourtium of 2 litres each and mineral medium of 2 litres. Sample of POME was initially analysed for COD, BOD, TSS, pH. Degradation process was performed for 24 hours and the sample was taken and analyzed every 3 hours for COD, BOD, TSS, pH and the bacterial growth.

2.5. Bacterial Growth and Parameter Analysis

Calculation of bacterial population was performed at each three hourse from 0 to 24 hours with total count method. The sample was mixed evenly. Ecopipette of 1 ml was used to take the sample and to place it to glass cover. The glass cover was placed in haemacytometer. There were 9 large rooms with area of 1 mm^2 and 1 large room in the middle with 25 medium rooms with the area of 0.2 mm^2 and one medium room contained 16 small rooms. The bacterial population was calculated at rooms of upper right, lower right, middle, upper left and lower left. (Nafi, 2015). The number of bacteria of the five rooms were averaged and it was multiply with 25 x 10^4.

3. Result and Discussion

3.1. Population Growth of the Bacteria

The bacterial growth of *Bacillus cereus* ATCC 14579 (KP 1.1), *Pseudomonas azotoformans strain NBRC 12693* (KP 1.3) and *Burkholderia cepacia* ATCC 25416 (KP 2.2) is shown in Figure 1. The bacterial groth increases along processing time for degradation of POME. The bacterial population increases during the processing time. The bacteril growth of *Bacillus cereus* ATCC 14579 (KP 1.1) increases more than other ones until the processing time reaches 12 hours. Higher growth of *Pseudomonas azotoformans strain NBRC 12693* (KP 1.3) followed by that of *Burkholderia cepacia* ATCC 25416 (KP 2.2) and *Bacillus cereus* ATCC 14579 (KP 1.1) from 18 to 24 hours of processing time. The bacterial consourtium of *Bacillus cereus* ATCC 14579 (KP 1.1), *Pseudomonas azotoformans strain NBRC 12693* (KP 1.3) and *Burkholderia cepacia* ATCC 25416 (KP 2.2) shows the highest growth of bacterial population from 15 to 24 hours as these bacteria work in synergism way to compose components of cellulose, protein and oil and fat in the POME.

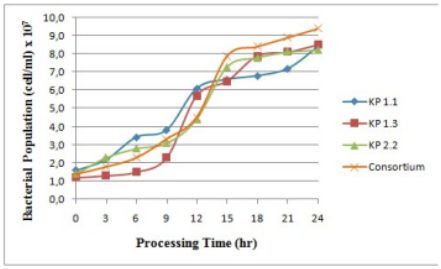

Figure 1. Bacterial Growth Curve

3.2. The Effect of Processing Time on BOD Values

Biological oxygen demand (BOD) represents dissolved and suspended organic compounds in POME. The highest BOD requires more dissolved oxygen to degrade the organic compounds. BOD decreases along the processing time as *Bacillus cereus* ATCC 14579 (KP 1.1), *Pseudomonas azotoformans*

strain NBRC 12693 (KP 1.3) and *Burkholderia cepacia* ATCC 25416 (KP 2.2) and the bacterial consortium degrade POME as shown in Figure 2. Reduction of BOD values of *Pseudomonas azotoformans strain NBRC 12693* (KP 1.3) and *Burkholderia cepacia* ATCC 25416 (KP 2.2) is not so differentiated but it is higher than that of *Bacillus cereus* ATCC 14579 (KP 1.1). Reduction of BOD values of the consortium of bacteria is higher than those of *Bacillus cereus* ATCC 14579 (KP 1.1), *Pseudomonas azotoformans strain NBRC 12693* (KP 1.3) and *Burkholderia cepacia* ATCC 25416 (KP 2.2) since it degrade the POME components simultaneously and BOD removal achieves 91.70 percent using the bacterial consortium.

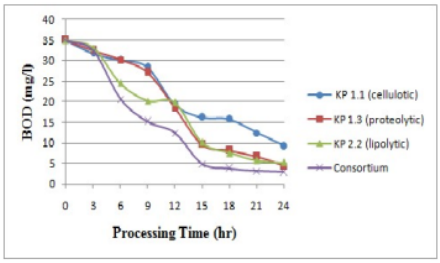

Figure 2. The Effect of Processing Time on BOD

3.3. The Effect of Processing Time on COD Values

COD values in POME indicates the organic and inorganic substances dissolved in it. COD values decreases along the processing time when degradation of the POME uses Bacillus cereus ATCC 14579 (KP 1.1), Pseudomonas azotoformans strain NBRC 12693 (KP 1.3) and Burkholderia cepacia ATCC 25416 (KP 2.2), as well as, the bacterial consortium. COD reduction is proportional to the bacterial growth. The bacteria consumes the organic and inorganic substances in the POME to form metabolites and the new bacteria. The highest reduction of COD is achieved at 82.31 percent for degradation of POME using the bacaterial consortium due to its capability as cellulolytic, proteolytic and lipolytic bacteria to degrade cellulose, protein, oil and fat, respectively.

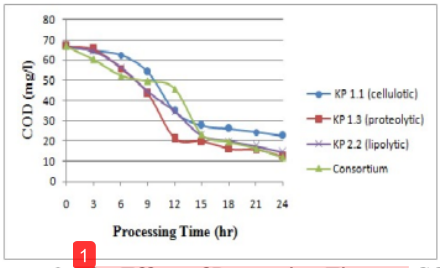

Figure 3. The Effect of Processing Time on COD

3.4. The Effect of Processing Time on TSS values

Total suspended solid (TSS) values in POME shows particles being suspended in the liquid waste. TSS values has correlation with COD and VOD values. The particle size is smaller than sediments with maximum size of 2 micrometres. COD values decrease along the processing time. The bacteria either individual or consortium reduce TSS values for the formation of metabolites and the new bacteria. The highest TSS removal reaches 73.75 percent as degradation results of POME using the bacterial consorsium.

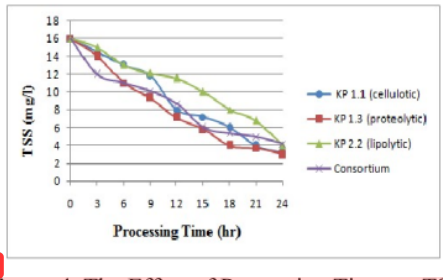

Figure 4. The Effect of Processing Time on TSS

3.5. The Effect of Processing Time on pH Values

The degradation of organic and inorganic substances is influenced by pH values of the liquid waste. Normal pH is required for aerobic digestion due to optimum performance of the bacteria in degrading the substanes in the POME. Values of pH during the processing time fluctuates as influenced by metabolites formed. The process of POME degradation can proceed well since the pH values ranging from 6.7 to 7.5 and it's range is still normal.

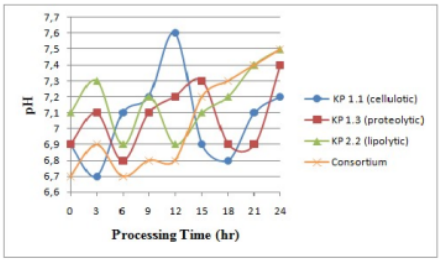

Figure 5. The Effect of Processing Time on pH

3.6. Growth Rate and Generation Time

Rates of growth of bacteria and generation time for *Bacillus cereus* ATCC 14579 (KP 1.1), *Pseudomonas azotoformans strain NBRC 12693* (KP 1.3), *Burkholderia cepacia* ATCC 25416 (KP 2.2) and the bacterial consortium are shown in Table 3 to Table 6, respectively. Rates of bacterial growth increase along the processing time and these declines after 15 hours of processing time. The highest growth rates for *Bacillus cereus* ATCC 14579 (KP 1.1), *Pseudomonas azotoformans strain NBRC 12693* (KP 1.3) and *Burkholderia cepacia* ATCC 25416 (KP 2.2) and the bacterial consortium are 0.159 hr^{-1} at 12 hour, 0.301 hr^{-1} at 12 hour, 0.167 hr^{-1} at 15 hour and 0.184 hr^{-1} at 15 hour, respectively. The generation time denotes time required to increase number of cells two folds of the initial one. The lowest generation times obtained for *Bacillus cereus* ATCC 14579 (KP 1.1), *Pseudomonas azotoformans strain NBRC 12693* (KP 1.3) and *Burkholderia cepacia* ATCC 25416 (KP 2.2) and the bacterial consortium are 6.300 hour, 3.322 hour, 5.975 hour and 5.422 hour, respectively. *Pseudomonas azotoformans strain NBRC 12693* (KP 1.3) denotes the most highest growth rates and the most lowest generation time among the other bacteria. Degradation of protein is more easier than that of cellulose and oil and fat that correlates to growth rates of the bacteria and generation time.

Table 1. Growth rates and generation time of *Bacillus cereus* ATCC 14579 (KP 1.1)

t (hr)	x (CFU/ml)	μ (hr^{-1})	g (hr)
0	1.6 x 10^7	-	-

3	2.2 x 10^7	0,101	9,903
6	3.4 x 10^7	0,147	6,795
9	3.8 x 10^7	0,036	28,061
12	6.1 x 10^7	0,159	6,300
15	6.6 x 10^7	0,027	37,675
18	6.8 x 10^7	0,008	117,932
21	7.2 x 10^7	0,020	49,769
24	8.4 x 10^7	0,050	19,924

Table 2. Growth rates and generation time of *Pseudomonas azotoformans strain NBRC 12693* (KP 1.3)

t (hr)	x (CFU/ml)	μ (hr^{-1})	g (hr)
0	1.2 x 10^7	-	-
3	1.3 x 10^7	0,035	28,769
6	1.5 x 10^7	0,049	20,598
9	2.3 x 10^7	0,137	7,313
12	5.7 x 10^7	0,301	3,322
15	6.5 x 10^7	0,042	23,780
18	7.9 x 10^7	0,066	15,136
21	8.1 x 10^7	0,007	139,026
24	8.5 x 10^7	0,016	62,573

Table 3. Growth rates and generation time of *Burkholderia cepacia* ATCC 25416 (KP 2.2)

t (hr)	x (CFU/ml)	μ (hr^{-1})	g (hr)
0	1,4 x 10^7	-	-
3	2,3 x 10^7	0,166	6,031
6	2,8 x 10^7	0,069	14,539
9	3,1 x 10^7	0,035	28,969
12	4,4 x 10^7	0,115	8,707
15	7,3 x 10^7	0,167	5,975
18	7,8 x 10^7	0,022	44,650
21	8,1 x 10^7	0,012	85,416
24	8,2 x 10^7	0,005	220,767

Table 4. Growth rates and generation time of bacterial consortium

t (hr)	x (CFU/ml)	μ (hr^{-1})	g (hr)
0	1,4 x 10^7	-	-
3	1,8 x 10^7	0,089	11,199
6	2,3 x 10^7	0,078	12,739
9	3,3 x 10^7	0,120	8,304
12	4,5 x 10^7	0,104	9,661
15	7,9 x 10^7	0,184	5,422
18	8,4 x 10^7	0,022	46,212
21	8,9 x 10^7	0,018	55,423
24	9,4 x 10^7	0,019	51,585

3.7. Kinetics of POME Degradation

Microbial kinetics of *Bacillus cereus* ATCC 14579 (KP 1.1), *Pseudomonas azotoformans strain NBRC 12693* (KP 1.3), *Burkholderia cepacia* ATCC 25416 (KP 2.2) and the bacterial consortium are obtained by using Monod Equation with modification of Langmuir Equation with COD as substrates. Value of μ_{max} = 0.067 1/hr, K_s = 111.767 mg/l, Y = 1.5x106 CFU/mg, q_{max} = 1.52x10-7 mg/CFU.day and K_d = 1.303 1/hr for *Bacillus cereus* ATCC 14579 (KP 1.1); μ_{max} = 0.057 1/hr, K_s = 91.603 mg/l, Y = $1.3x10^6$ CFU/mg, q_{max} = $2.99x10^{-7}$ mg/CFU.day and K_d = 1.912 1/hr for *Pseudomonas azotoformans strain NBRC 12693* (KP 1.3), μ_{max} = 0.042 1/hr, Ks = 78.848 mg/l, Y = $1.3x10^6$ CFU/mg, q_{max} = $1.8x10^{-7}$ mg/CFU.day and K_d = 1.277 1/hr for *Burkholderia cepacia* ATCC 25416 (KP 2.2) and μ_{max} = 0.394 1/hr, K_s = 267.88 mg/l, Y = $1.5x10^6$ CFU/mg, q_{max} = $2.6x10^{-7}$ mg/CFU.day and K_d = 2.121 1/hr for bacterial consortium. The value of μ_{max} achieved for the bacterial consortium is higher than those of *Bacillus cereus* ATCC 14579 (KP 1.1), *Pseudomonas azotoformans strain NBRC 12693* (KP 1.3) and *Burkholderia cepacia* ATCC 25416 (KP 2.2). Degradation of POME with the bacterial consortium is faster compared than *Bacillus cereus* ATCC 14579 (KP 1.1), *Pseudomonas azotoformans strain NBRC 12693* (KP 1.3) and *Burkholderia cepacia* ATCC 25416 (KP 2.2). The bacterial consortium degrades the components of cellulose, protein and oil and fat simultaneously in the POME.

4. Conclusion

The lowest generation time of KP 1.1 and KP 1.3 is achieved at 6.3 hours and 3,322 hour for 12 hour process degradation of POME with bacterial growth rate of 0.159 $hour^{-1}$ and 0.301 $hour^{-1}$ and thoses of KP 2.2 and bacterial consortium are achieved at degradation process of POME for 15 hour with the lowest generation time of 5.975 hour and 5,422 hour with the bacterial growth rates of 0.167 $hour^{-1}$ and 0.184 $hour^{-1}$. The highest reduction of BOD is 91.70 percent, COD 82.31 percent and TSS 73.75 percent with the consortium bacteria.

Acknowledgment

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