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Research Paper

Biodegradation of Methyl mercury by Bacteria of *Empedobacter brevis* in Leachate

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Abstract
Leachate treatment containing methyl mercury (CH_3Hg^+) can be done by using biodegradation method. Bacteria used in methyl mercury biodegradation of the bacteria *Empedobacter brevis*. This study aims to examine the ability of bacteria in degrading methyl mercury in leachate and determine the value of μmax and Ks. The degradation process is done with variation of inoculum concentration and incubation time aerobically. The parameters analyzed were the decrease of methyl mercury content in the biodegradation process. The results showed that biodegradation of methyl mercury by the bacterium *Empedobacter* brevis at 15% inoculum concentration was the highest decrease efficiency of 81%. The values of μmax and Ks in the bacterium *Empedobacter* brevis were 0.994 per hour with a substrate concentration of 1.588 mg /l. The reduction of methylmercury biodegradation process can be done by *Empedobacter brevis* on leachate.

Keywords
Leachate, methyl mercury, inoculum concentration, incubation time, bacterial growth rate and substrate concentration

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1. INTRODUCTION

Garbage becomes a serious problem in big cities in Indonesia along with increasing population and the limited land that will be used as Final Disposal Place (TPA). Dumps of waste that are left open in addition to causing odors also produce leachate. Leachate is formed by the seepage of rain water that goes down the pile of garbage. Leachate contains organic components, inorganic and heavy metals, one of which is mercury (Ali, 2011; Cabral et al., 2014).

Mercury is the only liquid metal compound at a normal temperature with a low vapor pressure (hua DING et al., 2007). Mercury derived from both domestic and industrial waste into the waters in the form of mercury (Hg^0) that is partially bonded in the sediment and partially suspended in water. UV radiation causes mercury (Hg^0) to undergo photolysis reaction into mercury ions (Hg^{2+}). Furthermore, mercury ions (Hg^{2+}) undergo methylation reactions with the help of microorganisms into methyl mercury compounds (CH_3Hg^+) (Li et al., 2015)

Methyl mercury (CH_3Hg^+) is the most toxic compound among other mercury species. These compounds accumulate in the sediment and enter the food chain. Methyl mercury accumulated in the food chain has high risk for top predators (Chai et al., 2015). Methyl mercury is a highly toxic compound because it can form lipophilic compounds that can cross the cell membrane, be absorbed easily and penetrate into the nervous system. Methyl mercury is easily formed under the anaerobic reduction conditions from the final waste dumps through the reduction activity of sulfate and iron bacteria (Chai et al., 2015).

The effort to prevent the presence of methyl mercury in leachate does not pollute the environment by doing biodegradation method. Bacteria *Empedobacter brevis* is one of the bacteria in degrading methyl mercury. *Empedobacter brevis* entered in the genus *Flavobacteriaceae* which is a short rod-shaped bacteria, non-motile, gram negative and aerobic. All strains grow at 30°C and there are several strains that grow at 37°C. This bacterium has a width of 0.5 μm and a length of 1-2 μm (hua DING et al., 2007). The purpose of this study is to assess the ability and determine the degradation kinetics of the bacteria *Empedobacter brevis* in degrading methyl mercury in leachate that is influenced by variation in the number of inoculum and incubation time.

2. EXPERIMENTAL SECTION

2.1 Materials

Lysogeny broth (LB), a nutritionally rich medium is mainly utilized for the growth of bacteria. The LB is also regularly, albeit incorrectly, taken to mean Luria broth, Lennox broth, or Luria-Bertani medium. In this study, we used Luria-Bertani broth which is from Darmstadt, Germany. Luria-Bertani Broth is the most common media to maintain and cultivate *Empedobacter brevis*. On the other hand, we analyzed methyl mercury by using toluene, acetone, HCl and NaCl.

2.2 Preparation of Bacteria Inoculum

The bacteria of Empedobacter brevis from the skewed medium were taken 2-3 ose aseptically placed into an erlenmeyer containing 50 ml of LB broth. Empedobacter brevis incubated for 24 hours at room temperature (±30°C) above shaker with agitation speed of 160 rpm.

2.3 Biodegradation Methyl mercury in Leachate

The bacterial inoculum was taken by the variation of bacterial concentrations around 15% (v/v), 20% (v/v), and 25% (v/v) were inoculated into leachate and incubated for 4, 8, and 24 hours with agitation speed 160 rpm at room temperature 30°C. Calculation of bacteria in substrate was done in every incubation time interval that is 4, 8 and 24 hours. The number of bacteria was calculated using Total Plate Count (TPC) method then the methyl mercury content was analyzed by Gas Chromatography - Mass Spectrometry.

The research design used was complete randomized design with factorial pattern, with combination of treatment as presented in Table 1.

2.4 Calculation Efficiency of Decreased Methyl Mercury Content

The efficiency of methyl mercury content is calculated with the following equation:

$$Eff = \frac{C_2 - C_1}{C_2} x100\% \quad (1)$$

Where: C_1 = Initial concentrations (mg/L) C_2 = Final concentration (mg/L) Eff = Efficiency (%)

2.5 Kinetics of Microbial Degradation

Bacterial degradation can be calculated kinetically to determine the value of bacterial growth rate (μmax) and substrate saturation constant value (Ks) by using Monod equation with Lineweaver-Burk method.

$$\mu = \mu m \frac{S}{Ks + S} \quad (2)$$

Notes : μ = Specific growth rate (min^{-1}) μm = Max specific growth rate (min^{-1}) S = Residual concentration of growth substrate (m/v) Ks = The saturation constant equals the substrate concentration (m/v)

3. RESULTS AND DISCUSSION

3.1 Empedobacter brevis Growth Curve

Growth is the regular addition of all components of a microorganism. The growth of microorganisms can be measured based on cell concentration or cell density (dry weight from cells of cell contents). Calculating cell density can be seen from the absorbance value of contents (Diana, 2013). The growth of microorganisms is an indicator that ca be decreased heavy metals in leachate. The growth of microorganisms will increase if it is able to live by utilizing the substrates present in the leachate.

Based on the measurement of absorbance with the number of colonies, the standard growth curve of Empedobacter brevis is Y = 3040.6 X - 1931.1, with correlation (r) = 0.9239. These standard curve equations can be calculated the number of Empedobacter brevis as seen in Figure 1.

The adaptation phase of Empedobacter brevis occurred at the 0th hour until the 2nd hour, while the exponential phase of Empedobacter brevis on each variation of methyl mercury concentration occurred at the 4th hour until the 28th hour. The stationary phase is characterized by the growth of bacterial cells that are almost constant or have very little growth of the bacteria Empedobacter brevis occurred at hours 28 to hours 56. The phase of death of the bacterium Empedobacter brevis occurred at the 56th hour.

3.2 The Empedobacter brevis Effects of Bacterial Concentration and Incubation Time in Leachate

Biodegradation research of methyl mercury in leachate used Empedobacter brevis with various inoculum concentrations or specific size. Giving various concentrations of this inoculum aims to determine which inoculum concentration is optimum to support bacterial growth and has a high absorption of methyl mercury.

The Influence of bacteria in certain amounts, especially bacteria that are adaptive, resistant to polluted media and can bind heavy metals because microbes produce extracellular or synthetic

Table 1. Table Combination Treatment

Bacterial Concentration	Incubation Time		
	B1 (4 hours)	B2 (8 hours)	B3 (24 hours)
A1 (15%)	A1B1	A1B2	A1B3
A2 (20%)	A2B1	A2B2	A2B3
A3 (25%)	A3B1	A3B2	A3B3

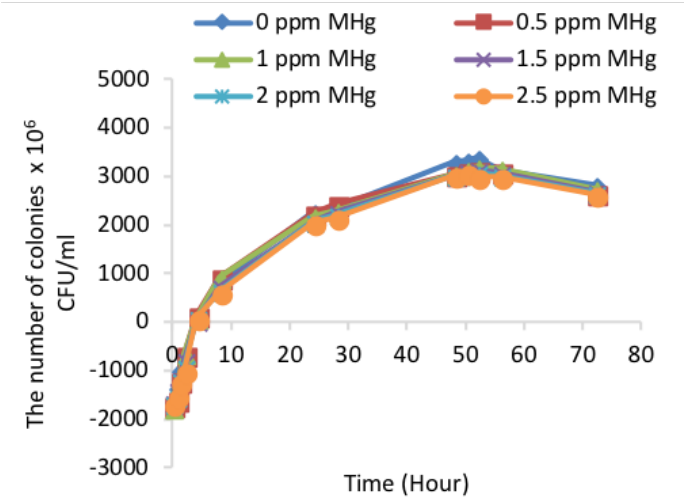

Figure 1. Growth of Empedobacter brevis in the variation of metilmercury concentration

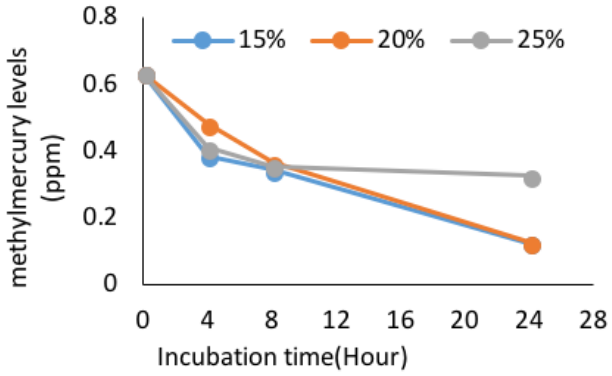

Figure 2. The influence of bacterial concentration and incubation time of Empedobacter brevis in leachate

enzymatic compounds capable binding heavy metals through adsorption processes (Hughes and R.K., 1989; Khoiroh, 2014). The biosorbtion mechanism was capable for living in a polluted environment of heavy metals. This mechanism occurs simultaneously with the consumption of metal ions for the growth of microorganisms (the intracellular accumulation of metal ions) (Khoiroh, 2014).

The number of microbes that increase can lead competition between microbes. This form of competition can be a fight for space, water and nutrient (Khoiroh, 2014). The competition between bacteria cause growth and degradation process will be low. The ability of biosorption from microbe is influenced by several factors such as group of gram positive and negative bacteria. Gram-negative bacteria are generally more tolerant of heavy metal influences than gram-positive bacteria because of their complex cell wall structures which can bind and immobilize most metal ions including CH_3Hg^+. The metals are bonded to carboxyl groups on the peptide and peptidoglycan chains and phosphate groups of lipopolysaccharides. Similarly, the presence of plasmids in bacteria can cause bacteria resistant to heavy metals (Hughes and R.K., 1989; Li et al., 2015).

The methyl mercury decrease with various treatments of inoculum concentration at incubation time 0, 4, 8 and 24 hours can be seen in Figure 2.

The initial methyl mercury was 0.633 ppm. The lowest decrease of methyl mercury occurred at 15% treatment around 0,124 at incubation time 24 hours which has efficiency decreased methyl mercury content around 81%. The bacteria concentration treatment at 20% was 0,128 with efficiency decreased methyl mercury content around 80%. The treatment at 25% inoculum concentration was 0,326 with efficiency decreased methyl mercury content around 48%. The results showed that the small concentration of bacterial inoculum in Empedobacter brevis improved the degradation process. The longer the incubation time, the lower the methyl mercury level exist in the leachate. The suitability between the inoculum concentration ratio and the substrate composition may affect the degradation process of methyl mercury. The decrease in methyl mercury levels at 15% and 20% inoculum concentrations did not differ greatly. So, the inter-population competition on the treatment resulted the bacteria adapting to use other substrates

Table 2. The values of kinetic parameters for methyl mercury biodegradation in leachate

Sample Code	Growth Rate, μ (Generation/Hour)	Generation Time, g (Hour)	μmax	Ks
A1B1	-	-	0,994	1,588
A1B2	0,126	7,936		
A1B3	0,016	62,5		
A2B1	-	-	0,347	0,868
A2B2	0,007	142,857		
A2B3	0,004	250		
A3B1	-	-	0,007	0,365
A3B2	0,033	30,303		
A3B1	0,002	500		

other than carbon (Sucipto, 2009).

3.3 Kinetics Degradation Methyl Mercury by Empedobacter brevis

Growth rate and generation time need to be calculated, because the relationship between growth rate and generation time to process time can be known in determining the optimum time. The optimum time of the process is marked by the lowest generation time and the highest biomass growth rates. The lowest generation time is the shortest time required to increase the number of cells becoming double from the original amount shown in Table 2. The biomass growth rate showed the bacterial population changes per unit of time, the low growth rate indicated the slow growth of microorganisms and vice versa. The lowest generation time and highest biomass growth rate for bacteria Empedobacter brevis at 15% inoculum concentration was the 8th hour that was the growth rate of bacteria was 0,126 generation per hour with time generation 7,936 hour. The highest generation time and the lowest biomass growth rate for the bacterium Empedobacter brevis at 25% inoculum concentration was the 24th hour with the bacterial growth rate around 0.002 generations per hour and the generation time around 500. The low growth rate signified the slow growth of microorganisms (Romli, 2012). Substrate concentration is a limiting factor of bacterial growth; a culture will achieve maximum biomass concentration as it approaches the end of the growth phase. The greater value of Ks indicated the substrate concentration was sensitive to the growth of microorganisms. The maximum growth rate (μmax) was the maximum value of the growth rate at the peak during the exponential phase before entering the stationary phase (Fahria and Laksmono, 2014).

The maximum growth rate of Empedobacter brevis bacteria was highest at 15% inoculum concentration of 0.994 per hour with a substrate concentration of 1.588 mg/L. The 20% inoculum concentration of 0.347 per hour showed substrate concentration around 0.868 mg/l and the lowest maximum at 25% inoculum concentration of 0.007 per hour indicated substrate concentration around 0.365 mg/L.

4. CONCLUSIONS

The highest efficiency of methyl mercury reduction in methyl mercury biodegradation process by Empedobacter brevis on leachate is at 15% inoculum concentration with a percentage decrease of 81%, this is the same with the kinetics value of bacterial biodegradation which has the bigger growth rate and the faster growth of microorganism. The highest maximum growth rate at 15% inoculum concentration is 0.994 generations per hour with a substrate concentration of 1.588 mg/L.

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