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BIODEGRADATION OF LEACHATE BY CONSORTIUM OF MICROORGANISM INDIGENOUS

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Abstract

Leachate is a liquid that occurs as a result of mixing rainwater runoff with rubbish that has decayed and contains very fine suspended substances and pathogenic microbes. Leachate can cause potential contamination for both surface and groundwater. In this study biodegradation of leachate by microorganisms was carried out to reduce the content of organic and harmful inorganic substances. The purpose of this study was to evaluate the potential biodegradation of bacterial isolates isolated from landfill leachate. The method used in this study is an experimental method with three repetitions. The parameters observed included the levels of Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Suspended Solid (TSS) and ammonia, for 14 days the biodegradation process. Data from the research results were analyzed using Variety Analysis (ANOVA) followed by Duncan's multiple distance test. The results showed that a consortium of Bacillus subtilis, Bacillus licheniformis, Bacillus pumilus, Pseudomonas putida, Nitrobacter and Nitrosomonas (K3) capable of reducing BOD levels by 68%, reducing COD levels by 89%, TSS by 71%, and Ammonia by 92 %, reducing 90 % of chrome and 90 % of lead.

Key words*: leachate, biodegradation, landfill, consortium.*

INTRODUCTION

Leachate is a liquid resulting from the decomposition of waste and rinsing by percolation by rainwater through landfills. Leachate defined as a liquid which filters through waste and decomposition results, and extracts dissolved material so that it suspended in the liquid. In another definition, leachate is a liquid waste formed by the entry of external water into landfills, dissolving and rinsing dissolved materials including organic and inorganic compounds resulting from the decomposition process (Meeroff and Lakner, 2014). Landfill utilization will always coincide with the production of leachate because the decomposition of waste and leachate occurs due to rain percolation which is the process of flowing water by gravity from the soil layer to the layer below it. Leachate washing will cause adverse effects such as pollution of groundwater and the environment and produce health risks for living organisms. Landfill leachate components include biodegradable and non-biodegradable products including organic matter, phenols, nitrogen ammonia, phosphate, heavy metals, sulfide, various heavy metal compounds.

In addition, leachate also contains pathogenic and non-pathogenic microorganisms.

In leachate, which also contains high ammonia nitrogen, the harmful impact of high ammonia nitrogen entering the waters is eutrophication which can cause algae blooms and siltation of waters.

The biodegradation process requires a long time to reach a high and active population. Bioremediation of leachate with bioaugmentation by the consortium of trading bacteria considered more economical and practical.

Because in the consortium, bacteria in high populations and various enzymes will produce which can work synergistically. A high population indicates that bacteria can use leachate as a nutrient. According to Doroty (2011), a number of species that can be found in leachate water include *Pseudomonas* sp*., Bacillus* sp*., Cellulomonas* sp*., Staphylococcus*

sp., *Acinetobacter* sp., *Actinobacillus* sp., *Alcaligenes* sp., *Klebsiella* sp., *Flavobacterium* sp., *Enterobacter* sp., *Serratia* sp., *Shigella* sp., *Moraxella* sp., *Pasteurella* sp., *Proteus sp., Yersinia* sp. The ability to degrade organic compounds is different because the enzymes produced are also different by each genus of microorganisms. Therefore, the use of consortium cultures on leachate bioremediation is considered more effective considering the variety of compounds contained in leachate.In this study biodegradation of leachate was carried out by using consortium isolates from leachate. This study aims to analyze the ability of the consortium of species of microorganisms to degrade leachate containing organic and inorganic materials. The parameters measured include Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) Total Solid Suspension (TSS), and Ammonium, Chromium (Cr) and lead (Plumbum; Pb) levels contained in leachate.

MATERIALS AND METHODS

Materials: Nutrient Agar (NA), sulfuric acid, starch indicators, Na-thiosulfate, Kl, $MgCl_2$, $FeCl_3$, sulfate buffer, H_2SO_4, and $MnSO_4$; Ag_2SO_4, distilled water, 0.01N $KMnO_4$, H_2C2O_4, hydrogen peroxide (H_2O_2).

Methods: The method used in this study is the experimental method with Completely Randomized Design (CRD), the factorial pattern of the AxB with three repetitions. The factor I is a different bacterial consortium. Factor II is the length of biodegradation time. Each treatment repeated three times. The consortium used is: (k0): Leachate; (k1): Leachate inoculated by *Bacillus subtilis*, *B. licheniformis*, and *B. Pumilus*; (k2): Leachate inoculated by *B. subtilis*, *B. licheniformis*, *B. pumilus*, and *Pseudomonas putida;* (k3): Leachate is inoculated by *B. subtilis*, *B. licheniformis, B.pumilus, Pseudomonas putida*, *Nitrosomonas* sp., and *Nitrobacter* sp.

The parameters measured were the number of bacteria, pH, levels of Biochemical oxygen demand (BOD) (SNI 6989.72.2009, BSN, 2004), Chemical oxygen demand (COD) (SNI 6989.2.2009,BSN, 2004) Total solid suspension (TSS) (SNI 06-6989.3-2004,BSN, 2004), Total plate counts andammonia(SNI 06-6989.30-2005, BSN, 2005) Chromium and Pb levels as measured by Atomic Absorption Spectroscopy (AAS). Biodegradation of leachate is carried out for 14 days. The data obtained were tested statistically using variance analysis (ANOVA).

RESULTS AND DISCUSSIONS

Growth of bacteria during the biodegradation process

The number of bacteria that grows in the biodegradation process is calculated using the Total Plate Count (TPC) method every 48 hours for 14 days. Bacterial growth during the biodegradation process was analyzed using variance analysis (ANOVA) and Duncan's Multiple Distance Test. ANOVA results and Duncan's Multiple Distance Test in Table 1. show that all consortiums can grow and use nutrients found in leachate. However on the sixth day, the bacteria grows and reaches the maximum population and subsequently decreases the population. The high population of bacteria found in the *B. subtilis* consortium, *B. licheniformis, B. pumilus, P. putida, Nitrosomonas* sp., and *Nitrobacter* sp. which shows that bacterial species in the consortium grow synergistically.

Bacillus generally has relatively fast growth which causes *Bacillus sp*. widely used in the biodegradation process. Landfill leachate characterized by high organic and inorganic pollutant concentrations and is extremely toxic to the environment.

The constituent in landfill leachates include organic materials such as aromatic groups, chlorinated aliphatic, phenols, phthalates, pesticides, and even ammonia, inorganic salts, such as Chromium, lead and copper, are acidic, but also extremely high concentrations of ammonia and organic nitrogen (Purwanta and Susanto, 2017; Mukherjee et al., 2014).

Tabel 1. Bacterial growth (log10 CFU / ml) during leachate biodegradation process

Consortium	Time of **Biodegradation (days)**							
	0	2	4	6	8	10	12	14
Control	0 a A	5.33 b A	7.30 c A	10.3 g A	10.0 fg A	9.6 f A	8.06 d A	8.7 e A
B. subtilis, B. licheniformis, B. pumilus	0 a A	9.53 b C	12.6 d BC	22.0 g B	15.8 f B	11.7 c C	9,8 b B	15.1 d D
B. subtilis, B. licheniformis, B. pumilus, P. putida	0 a A	9.10 b B	12.4 e B	24.8 h C	14.3 g C	11,5 d C	10.0 c BC	13.8 f C
B. subtilis, B. licheniformis, B. pumilus, P. putida, Nitrosomonas, Nitrobacter	0 a A	11.46 d D	19.2 g C	30.6 h D	15.8 f C	10.6 c B	11.26 e C	9.6 b B

Microorganism-induced degradation of organic materials depends on the activity of various hydrolytic enzymes. Bacillus genus such as *Bacillus licheniformis, B. cereus, B. subtilis, B. coagulans, B. pumilus, B. smithii, B. brevis* produces various enzymes such as cellulase, proteases, amylase to decompose various components contained in waste. Pseudomonas putida produces several enzymes, including lipase, chitinase, and xylanase (Schallmey et al., 2004; Inca-Torres et al., 2018). According to Sunar et al., (2014) *P. putida* is an effective biodegradation agent and can multiply in waste. The Bacillus genus has interesting physiological properties because each species has different abilities, including being able to degrade organic compounds such as proteins, starch, cellulose, hydrocarbons, and dyes, produce antibiotics, play a role in nitrification and denitrification, nitrogen binding, selenium oxidizing, oxidizing and reducing manganese (Mn) (Bhatnagar and Kumari, 2013). Nitrosomonas and Nitrobacter are chemoautotrophic organisms found in soil and water, and are responsible for the oxidation of ammonium to nitrite (Nitrosomonas) and nitrite to nitrate (Nitrobacter) or called nitrification.

Reduction of Biochemical Oxygen Demand (BOD)

Biological Oxygen Demand (BOD) shows the amount of dissolved oxygen needed by living organisms to break down or oxidize waste materials in water. BOD examination is required to determine the pollution load and to design a biological treatment system. Decomposition of organic waste through the process of oxidation by microorganisms in water is a natural process that readily occurs when the wastewater contains sufficient oxygen.In sewage, organic pollutants are naturally described by existing bacteria. If there is enough oxygen, the bacteria will decompose aerobically, but if the bacteria runs out of oxygen, the decomposition will be carried out by anaerobic bacteria.

Table 2. Decrease in BOD during the biodegradation process (mg/l)

Treatment	**Initialof BOD (mg/l)**	**Duration of Biodegradation**		**Reduction**
		Day 0	Day 14	**(%)**
Leachate without the addition of microorganisms	940,00	940,00 a A	805,00 b A	14%
Consortium of *B.subtilis + B.licheniformis + B.pumilus*	940,00	937,33 a A	473,33 b AB	49%
Consortium of B.subtilis + B.licheniformis + B.pumilus + P. putida	940,00	935,00 a A	461,33 b B	50%
Consortium *B.subtilis + B.licheniformis + B.pumilus + P.putida + Nitrosomonas + Nitrobacter*	940,00	932,00 a A	301,00 b C	67%

Note:
The same lowercase letter read horizontally shows no significant difference (p> 0.05)
The same capital letter read towards the vertical shows that it is not significantly different (p> 0.05)

From the results of BOD measurements (Table 2), biodegradation of BOD by *Bacillus subtilis* consortium, *Bacillus licheniformis, Bacillus pumilus, Pseudomonas putida*, *Nitrosomonas*, was able to reduce BOD levels more than other consortiums namely from BOD 940 mg/l to 301.00 mg/l or 67% within 14 days. The only biodegradation by the genus Basilus decreases only 49%. Biodegradation with the consortium is more beneficial because it is synergistic because it produces various enzymes that can decompose the organic matter contained in

leachate. *Bacillus licheniformis* produces amylase; *Bacillus subtilis* produces amylase and protease. *Bacillus pumilus* produces cellulase, and *Pseudomonas putida* produces lipases (Thakur, 2012). According to Safitri, et al. (2015) *B. pumilus* is an ammonia oxidizing bacterium. The synergistic ability of the consortium of *Bacillus*, *Pseudomonas*, *Nitrosomonas* and *Nitrobacter* species on leachate bioremediation resulted in effective leachate degradation ability. Reduction of Chemical Oxygen Demand (COD) levels during the biodegradation process.

Chemical Oxygen Demand (COD) COD is the amount of oxygen needed to oxidize organic substances contained in liquid waste by utilizing potassium dichromate oxidizer as a source of oxygen.

Table 3. COD levels in the biodegradation process by three types of consortium (mg/l)

Treatment	Initialof BOD (mg/l)	Duration of Biodegradation		Reduction (%)
		Day 0	Day 14	
Leachate without the addition of microorganisms	3050.00	3050.00 a A	2220.00 b A	27%
B.subtilis, B.Licheniformis, B.pumilus	3050.00	3000.00 a A	849.0 b B	71%
B.subtilis, B.licheniformis, B.pumilus Pseudomonas putida	3050.00	3010.00 a A	667.0 b B	77%
B.subtilis + Bacillus licheniformis + Bacillus pumilus + Pseudomonas putida + Nitrosomonas + Nitrobacter	3050.00	3015.00 a A	576.0 b B	80%

Note:
The same lowercase letter read horizontally shows no significant difference (p> 0.05)
The same capital letter read towards the vertical shows that it is not significantly different (p> 0.05

COD is a measure of water pollution by organic substances that can naturally be oxidized through biological processes and can cause reduced oxygen dissolved in water.

The results of COD analysis during the biodegradation process in Table 3 show that the *Bacillus subtilis* consortium, *Bacillus licheniformis, Bacillus pumilus, Pseudomonas putida*, *Nitrosomonas* and *Nitrobacter* were able to reduce COD levels higher than other consortiums namely from COD levels 3015 mg/l to 576 mg/l or 80%. All consortia can reduce COD levels. This decrease shows that every bacterial species in the consortium produces enzymes that can degrade organic elements in leachate. Microorganisms in the consortium use organic ingredients as nutrients for their growth. According to Ajao et al., (2011) research, *Bacillus subtilis* was able to reduce COD levels of waste by 86% within 15 days. *Bacillus* sp. Isolated from areas contaminated with oil spills can produce proteases and lipases (Lee et al., 2015). *Nitrosomonas* sp. convert ammonia compounds into nitrite compounds, and *Nitrobacter* converts nitrite to nitrate. Therefore biodegradation by species consortium is more effective because it will describe the diversity of pollutant compounds in leachate.

Total Suspended Solid (TSS)

Total Suspended Solid (TSS) are solid substances that are suspended in waters and cause turbidity in the waters.

TSS values during the process of biodegradation of leachate by the consortium of *B. subtilis, B. licheniformis, B. pumilus, P. putida*, *Nitrosomonas* and *Nitrobacter* (Table 4) showed that the consortium was able to reduce TSS levels compared to other consortiums, TSS levels of 1005 mg/l to 329 mg/l or decreased by 67%. This result showed that bacterial species in the consortium could use organic matter in their waste as nutrients.

The *Bacillus* genus can decompose crude fibers and lignin which are difficult to decompose and hydrolyze cellulose so that solid organic matter dissolved in waste in the form of lignin, lipids, and cellulose can reduce. *Bacillus pumilus* has the high cellulolytic ability, so dissolved solids containing cellulose can be broken down. The consortium of *B. pumilus, B. subtilis, P. amylolyticus* and *Nitrosomonas* sp. Bacteria resulted in the highest reduction in TSS levels of 85% for 20 days (Zaira, 2014). This result showed that bacterial consortium interaction synergistically so that bacteria can utilize and decompose the substrate of organic matter in leachate.

According to Safitri et al. (2015), reduced suspended solids caused by the degradation of organic compounds by bacterial enzymes of degrading bacteria, during the biodegradation process. The ability of bacteria to decompose organic matter causes the suspended solids to decrease, and the value of TSS is also lower.

Table 4. Levels of total suspended solid (TSS) (mg/l) during the process of biodegradation of leachates by three consortium types

Treatment	Initial of BOD (mg/l)	Duration of Biodegradation		Reduction (%)
		Day 0	Day 14	
Leachate without the addition of microorganisms	1112,00	1112.00 a A	1059.00 a A	4%
B.subtilis, B.Licheniformis, B.pumilus	1112,00	1010.00 a A	550.0 b B	45%
B.subtilis, B.licheniformis, B.pumilus Pseudomonas putida	1112,00	1020.00 a A	407.0 b B	60%
B.subtilis + Bacillus licheniformis + Bacillus pumilus + Pseudomonas putida + Nitrosomonas + Nitrobacter	1112,00	1005.00 a A	329.0 a B	71%

Note:
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The same capital letter read towards the vertical shows that it is not significantly different (p> 0.05).

Ammonia

Ammonia concentration is very high in leachate landfills due to the accumulation of organic waste including protein - ammonia produced from the decomposition of nitrogen-containing organic compounds and hydrolysis of urea in wastewater. Ammonia arises from the ammonification process, namely the process of utilizing organic compounds from dead living things such as proteins and amino acids by decomposing bacteria, also, ammonia can also derive from organic nitrogen originating from urine and livestock feces or excreta poultry. Fertilizers, feed, and various organic materials are sources of ammonia. According to Mpenyana et al., (2008), ammonia concentrations (> 10 mg N L^{-1}) produce some problems including eutrophication in waters because N is a vital growth nutrient for plants. Leachate contaminates water underground, when entering the river, and the lake will affect aquatic animals, which is due to reduced oxygen dissolved in the receiving water body, ammonia will be oxidized to nitrite, nitrite to nitrate and nitrate will be converted into nitrogen gas by denitrification bacteria. Ammonia and nitrite are toxic to fish, shrimp, and other aquatic fauna.The results of the ammonia concentration (Table 5) showed that the *Bacillus subtilis* consortium, *Bacillus licheniformis, Bacillus pumilus, Pseudomonas putida,* Nitrosomonas and Nitrobacter were able to reduce Ammonia levels greater than other consortiums namely Ammonia levels 1597 mg / l to 115 mg / l or equal to 92% on day fourteen. The decrease in ammonia is due to the presence of *Nitrosomonas*, and *Nitrobacter* as nitrifying bacteria. Nitrifying bacteria get nutrients from the decomposition of organic compounds into simpler compounds by bacterial species in the consortium, so that they can work more effectively to reduce ammonia in leachate.

Table 5. Ammonia levels during the biodegradation process

Treatment	Initialof BOD (mg/l)	Duration of Biodegradation		Reduction (%)
		Day 0	Day 14	
Leachate without the addition of microorganisms	1597	1597 a A	1330,00 a A	16%
B. subtilis, B. licheniformis, B. pumilus	1597	1597 a A	1030 b A	35%
B. subtilis, B. licheniformis, B. Pumilus, Pseudomonas putida	1597	1597 a A	516 b B	67%
B. subtilis + Bacillus licheniformis + Bacillus pumilus + Pseudomonas putida + Nitrosomonas + Nitrobacter	1597	1597 a A	115 b B	92%

Note:
The same lowercase letter read horizontally shows no significant difference (p> 0.05)
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Ability to bind heavy metals

Heavy metals often found in leachate are lead (Pb) and chromium (Cr). Heavy metal lead (Pb) and chromium (Cr) contained in leachate derived from waste that has disposed of to landfill (TPA). The highest decrease in heavy metal content occurs after day 14 of the consortium can be seen in Figures 1 and 2.

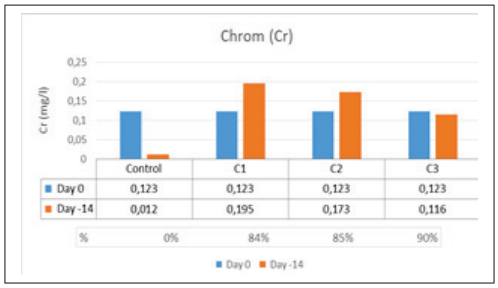

Figure 1. Decrease in Chrom (Cr) during the biodegradation process of leachate for 14 days

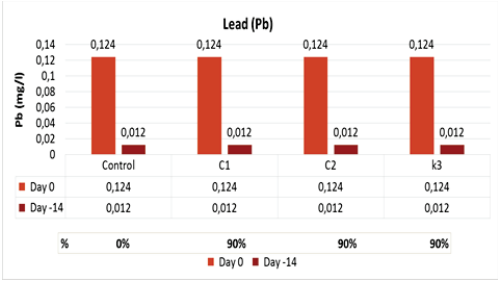

Figure 2. Decrease in Lead (Pb) during the biodegradation process of leachate for 14 days

The reduction of Chrom and Lead, heavy metals (Figure 1 and Figure 2) was carried out by all consortia, but *Bacillus subtilis* consortium, *Bacillus licheniformis, Bacillus pumilus, Pseudomonas putida*, *Nitrosomonas*, and *Nitrobacter* can reduce Cr 90% and 90% Pb respectively. Microorganisms have a strategy to tolerate heavy metals by transforming dangerous elements into safe forms, binding intra-extracellular metals and actively transporting metals out of the cytosol cells (Monachese et al., 2012).

According to Agostinho (2012), *Bacillus* sp. and *P. aeruginosa* can reduce 66% Cr content found in hospital waste. The mixture of *Pseudomonas pseudomallei* and *Pseudomonas aeruginosa* bacteria can reduce the highest levels of lead metal (Pb) in treatment with a decrease of 65% (Khoiroh, 2015). *Bacillus subtilis* was able to interact with a range of toxic metals, including copper, iron, magnesium, gold, and leads - charge of bacteria and cationic charge of many metals.

Gastrointestinal microorganisms also provide the body's first defense by converting toxic Cr (VI) to a less-toxic Cr (III). There are three types of mechanisms for binding heavy metals to bacterial cell walls, namely through (i) ion exchange reactions with peptidoglycan and teichoic acid, (ii) precipitation through nucleation reactions, and (iii) complexation with nitrogen and oxygen ligands. Gram-positive bacteria, particularly Bacillus spp., Have high adsorptive capacity due to high cholesterol and acid content in their cell walls of cells (Monachese et al., 2012).

CONCLUSIONS

The most effective consortium for degrading leachate is *Bacillus subtilis* consortium, *Bacillus licheniformis, Bacillus pumilus, Pseudomonas putida*, *Nitrosomonas*, *Nitrobacter* (C3) can reduce BOD by 67%. The level of COD reduction was 89%, reducing TSS levels by 71%, reducing ammonia levels by 92%, decreasing Cr and Pb levels by 90%.

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