Comparison of Enzymes Production of Bacteria from Landfill Soil and Leachate: A Case Study-Jabor Landfill Kuantan, Pahang, Malaysia

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***Abstract*—Jabor landfill commonly known as Kuantan landfill receives more than 500 tons per day waste with a composition of 60% of domestic waste and 40% of industrial waste. Landfill system always produce leachate. This waste contains many types of bacteria which potential to degrade the waste compound. Samples were taken from different places, landfill soil and leachate. Bacteria found in landfill soil were 19 different species of bacteria which are three Gram positive and 16 Gram negative bacteria. Bacteria were found in leachate tank were found to be 18 different species of bacteria. Those are five Gram positive and 13 Gram negative bacteria. Almost all of the bacteria in the soil and landfill leachate are able to produce amylase and protease enzymes, but only a few bacteria produce the lipase enzyme. The best bacteria are *Bacillus amyloliquefaciens, Bacillus licheniformis, Bacillus subtilis, Bacillus ruris and Kocuria varians*.**

***Index Terms*—Landfill, leachate, *Bacillus*, Gram negative and Gram positive bacteria.**

I. Introduction

Jabor jerangau Landfill is located in Pahang Service Area 1 (PSA1) catering the District of Kuantan in the State of Pahang. Approximately 300 km from Kuala Lumpur and 25 km from the City of Kuantan. Total site area is 60 hectares, divided into 3 sections. Parcel A covers an area of 27 ha for future expansion, parcel B covers 16 ha in phase 1 & 3, parcel C area of 16 ha was still in phase 2. Phase 2 (level 3 upgrade) and phase 3 is the sanitary cell. Here, there is also a system of leachate containment, leachate collection system, leachate treatment plant and landfill gas management [1].

Variable which is the most dominant in the municipal solid waste flow is food waste. Relatively homogeneous residential waste, some differences in the waste depends on local factors and other demographic, most households dispose of the same type of waste [2]. Trends in the composition of MSW in Malaysia showed that the food, paper and plastic are the main component of waste generated in most places [3]. The main householdwaste composition includes 71% organic waste, 12% plastic, 7.5% paper and paper products, 5% dirt and construction debris and 1% hazardous waste. The highest percentage is organic waste, although the composition of the waste varies depending on the source. Analysis of the composition of the waste comes from restaurants, hotels, schools and roads [4].

Leachate production is one of the biggest problems associated with the operation of environmentally sound sanitary landfill, because the liquid waste can cause harmful pollution problems by contaminating surfaces and ground water, surrounding soil surfaces. Clostridium perfringens, and fungal filaments are usually contaminated leachate. In addition there are bacteria, which includes aerobic, coliform and fecal coliform, psychrophilic and mesophilic bacteria, and spore-forming bacteria [5]. Two bacterial groups for almost the entire duration of treatment showed a good adaptation and critical participation of the two groups in the leachate treatment is Actinomycetes and Bacillus [6].

There are only two species of Gram-negative and six Gram-positive that can be isolated from both the leachate and bulk materials, and none of yeast Candida sp. or Cryptococcus sp. isolated from solid samples found in the leachate. Analysis of individual substrate utilization patterns of bacteria isolated from the leachate collected at successive sampling dates showed a decrease in the percentage of Gram-negative bacteria are able to metabolize sugar selected with increasing the percentage of Gram-positive bacteria capable of to metabolize them [7]. Therefore, this study is to compare the number of species of bacteria that live in soil and landfill leachate which potential to produce amylase, protease and lipase enzymes. The bacteria could be the agent of biodegrader for municipal solid waste treatment.

II. Material and Methods

A. Sampling for Microbial Isolation

Soil samples and leachate were collected from Jabor-Jerangau landfill. The location of the landfill is at 30 56' 53" N, 1030 21' 03" E, along Jabor-Jerangau Road, District of Kuantan, State of Pahang, Malaysia. Kuantan Jabor-Jerangau Landfill (known as Kuantan Landfill) was first opened in 1993 as the designated landfill for municipal solid waste for the Kuantan town, Pahang, Malaysia. Soil samples were taken at depths of 0-20cm. Leachate samples were taken from the leachate tank. All samples were kept in a temperature of 4^{o} C.

B. Isolation of Bacteria

One gram of soil sample was added into 99 ml of distilled water. Then, 1 ml from the sample was taken out and added into 9ml of distilled water. This step was continuously

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repeated until 9th dilution, 0.1 ml from each dilution tube was spread on the nutrient agar plate. For each plate, L stick was used to spread the dilution on the medium. The plates was labelled and kept in the 37 ℃ in incubator for 24 hour.

C. Enrichment, Purification and Morphological Test of Bacteria

Isolation of these microorganisms until get single colony had been done by using serial dilution method and streaking method. After nutrient agar plate was ready, the fast growing of bacteria plate is taken. Plates were incubated to counting of colony forming units (cfus) to determine the average number of cfus per milliliter of leachate or wet weight g of bulk sample. The different shape of bacteria on the plate is chosen. By using aseptic technique, the selective bacteria on the agar plate are streaked. The streak agar plate is sealed and kept in 37 ℃ incubator for growing. After 24 hours, purification of bacteria was done doing by another streak technique and incubation. When the bacteria were pure culture, the next step is gram staining under microscope. The cocci and the rods were counted and identified following Gram staining and microscopic observation of the CFU isolated in Nutrient Agar. All the microorganism cultures were maintained at 4^{o}C in nutrient agar as stock and were subcultured at 15 days interval.

D. Screening for Enzyme Production of Bacteria

Meat Extract 3g (g/L), peptic digest of animal tissue 5g (g/L), starch soluble 2g (g/L), agar 15g (g/L). Final pH (at 25 ℃) 7.2 ±0.1. Heat to boil and to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121 ℃) for 10 minutes. Mix well and pour in sterile petri plates. The positive result of Amylase production show clear zone around the colonies after flooding by iodine.

Skim milk powder 28g (g/L), casein enzymic hydrolysate 5g (g/L), yeast extract 2.5g (g/L), dextrose 1g (g/L), agar 15g (g/L). Final pH (at 25 ℃) 7.0 ±0.2. Heat to boil and to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121 ℃) for 15 minutes. Mix well and pour into sterile Petri plates. Proteolytic bacteria hydrolyze casein to form soluble nitrogenous compounds was indicated as clear zone surrounding the colonies.

Agar plate containing rhodamine B 0.001 % (w/v), nutrient broth 0.8 %, NaCl 0.4 % (w/v), agar 1 % (w/v) and olive oil 2 % was prepared in distilled water, adjusted pH of 6.5. The assay incubated at 55 °C for 18 h and lypolitic activity was identified as an orange halo around colonies under UV light at 350 nm.

E. Identification of Bacteria

The best isolated bacteria that able to produce amylase, protease and lipase enzymes were identified using the BIOLOG Omnilog Gen III microbial identification system with Omnilog Gen III Operating Software.

III. Result and Discussion

A. Isolation and of Bacteria

Thirty seven isolates were found from three different sites (Solid soil, wet soil and leachate). Bacteria with different physical characteristics will be transferred to a new plate with a streaking method to find a single colony and pure culture. The isolation showed estimates colony forming units. According to the concentration of each dilution, the smaller dilution rate, the greater the number of colonies of bacteria. Bacteria found in solid soil were more numerous than leachate.

Estimated total colonies derived from landfill soil were approximately 69×10^{4} to 20×10^{9} CFU/ml. While total colonies derived from leachate were approximately 12×10^{4} to 1×10^{9} CFU/ml. From Table I, population of bacteria from landfill soil more numerous than bacteria from leachate. It is caused the condition of leachate which is containing toxic material, heavy metals and oxygen limited availability make only a few specific bacteria could thrive. After determining the number of colonies that grow, the next step is to move a different bacteria to new petridish to obtain single colony separate with streaking method. From 3 times streaking, the expected growing bacteria were pure culture and contained a single colony which would then be tested by the method of Gram Staining.

TABLE I: Population of Bacteria

No	Dilution Factor	Soil	Leachate
1	10^{-3}(1)	30×10^{4} CFU/ml	12×10^{4} CFU/ml
2	10^{-3}(2)	69×10^{4} CFU/ml	2×10^{4} CFU/ml
3	10^{-7} (1)	11×10^{8} CFU/ml	1×10^{8} CFU/ml
4	10^{-7} (2)	19×10^{8} CFU/ml	1×10^{8} CFU/ml
5	10^{-9} (1)	60×10^{9} CFU/ml	1×10^{9} CFU/ml
6	10^{-9} (2)	20×10^{9} CFU/ml	1×10^{9} CFU/ml

B. Gram Characteristic and Screening of Enzyme Production

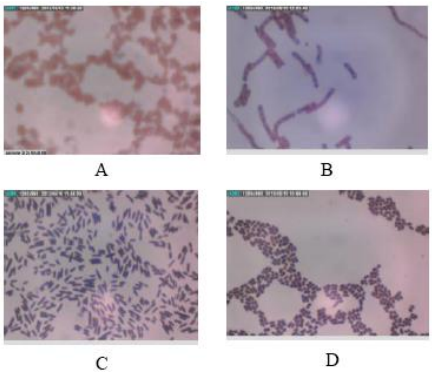

Fig. 1. Different colour and morphology of bacteria (A. staphylococci; B. streptobacilli; C. bacilli; D. diplococci).

Gram staining consists of four components. Namely, primary stain (crystal violet), mordant (lugol's iodin), decolourizer (ethanol) and counterstain (safranin). Many theories explain why some bacteria were able to retain the dye and the other does not. For example, the theory of differences in cytoplasmic pH, and the presence of magnesium ribonucleic on Gram positive and Gram negative does not exist on the widely unknown. Moreover, the thickness of the cell wall of Gram-positive and more lipid content in the cell wall of Gram-negative is a more acceptable reason for Gram stain reaction. Other factors are decolorized

process. When over-Decolorized, even Gram-positive bacteria may appear pink and when under-Decolorized gram-negative bacteria may appear Gram-positive. Gram reaction also depends on the age of the cell. Then, the glass slide observed under a microscope and oil immersion. After decolorization, the Gram-positive cell remains purple and the Gram-negative cell loses its purple color. Counterstain which is applied last to give decolorized Gram-negative bacteria a pink or red color (Fig. 1).

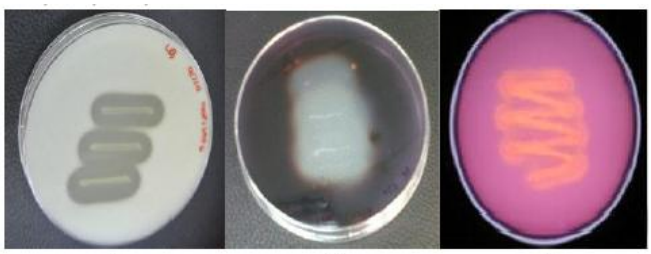

Fig. 2. Screening of enzyme production in selective media Skim milk agar; B. Starch agar; C. Rhodamine B agar).

TABLE II: MORPHOLOGY, GRAM CHARACTER AND ENZYME PRODUCTION OF BACTERIA FROM LANDFILL SOIL

No	Code	Morphology	Gram Character	Enzyme Production		
				Amylase	Protease	Lipase
1	TB	Bacilli	Positive	+++	++	-
2	TA	Bacilli	Negative	+	+	-
3	TC	Bacilli	Negative	+++	+	-
4	TD	Bacilli	Negative	+++	+++	+
5	TE	Staphylococci	Negative	-	+	+
6	TF	Bacilli	Negative	+++	++	-
7	TG	Streptobacilli	Positive	++	++	-
8	TH	Staphylococci	Negative	-	+	-
9	TI	Tetrad	Negative	++	+	-
10	TJ	Streptobacilli	Negative	+++	+	-
11	TK	Bacilli	Positive	+++	+	+
12	TL	Bacilli	Negative	-	-	-
13	TM	Bacilli	Negative	+++	+++	-
14	TN	Streptobacilli	Negative	+++	++	-
15	TO	Bacilli	Negative	+++	+++	-
16	TP	Bacilli	Negative	+++	+	-
17	TQ	Bacilli	Negative	+++	++	-
18	TR	Bacilli	Negative	+++	-	-
19	TS	Bacilli	Negative	++	+	-

TABLE III: MORPHOLOGY, GRAM CHARACTER AND ENZYME PRODUCTION OF BACTERIA FROM LEACHATE

No	Code	Morphology	Gram Character	Enzyme Production		
				Amylase	Protease	Lipase
1	LA1	Diplococci	Negative	-	-	-
2	LA2	Diplococci	Negative	++	-	-
3	LA3	Diplococci	Negative	-	-	-
4	LB1	Bacilli	Negative	-	-	-
5	LB2	Bacilli	Negative	-	-	-
6	LCA	Bacilli	Positive	-	+	-
7	LD1	Streptobacilli	Negative	++	++	+
8	LD2	Streptobacilli	Negative	++	++	+
9	LE	Bacilli	Negative	-	+	-
10	LF	Diplococci	Negative	-	-	-
11	La	Diplococcus	Negative	+	-	-
12	Lb	Bacilli	Negative	+	-	-
13	Lc	Staphylococci	Positive	++	-	-
14	Ld	Bacilli	Positive	-	++	-
15	Le	Bacilli	Positive	+++	+	-
16	Lf	Bacilli	Negative	+	+	-
17	Lg	Bacilli	Positive	++	+	-
18	Lh	Streptobacilli	Negative	-	+	-

Bacteria were found to be 19 different species of bacteria in soil of Jabor landfill (Table II). Three Gram positive and 16 Gram negative bacteria. The positive result of enzyme production (Fig. 2) in selective media show clear zone around the colony for skim milk agar which indicated protease activity. Halo zone showed in starch agar after flooding by iodine indicated that the bacteria produce amylase. Lipolytic activity in rhodamine B agar showed the

orange halo under UV lamp 350nm. Almost all bacteria produce amylase and protease, just few bacteria could produce lipase. Proteases as a good indicators of organic matter decomposition on account of their dependence on substrate, also act on proteins and polypeptides degradation. Bacteria convert starch molecules to glucose by producing amylases enzyme [8]. Therefore, from landfill soil there were TD, TE, TK, TM and TO as the best bacteria.

Bacteria were found in leachate tank of Jabor landfill were found to be 18 different species of bacteria. Five Gram positive and 13 Gram negative bacteria (Table III). Among the Gram positive and Gram negative bacteria isolated, rods were more frequently isolated than cocci of both samples. The Gram positive bacteria identified in leachate included two species of *Bacillus*. *B. brevis* and *B. megaterium*., one species of *Cellulomonas cellulans*., and one species of *Staphylococcus delphini*. Species in the first two genera have been previously identified in landfill material. The microbiological analyses indicate that four bacterial groups were responsible for the biological treatment in leachate, Tunisia were *Actinomycetes, Bacillus, Pseudomonas* and *Burckholderia* [6]. Activity of enzyme production of leachate samples less than the landfill soil because the content of starch, protein and lipid in landfill soil more than in leachate. The best bacteria from leachate were LD1 and LD2.

C. Identification of Bacteria

Several bacteria found were pathogenic bacteria in the different waste samples. *Escherichia* were present in all samples, but distinction between strains of the normal intestinal flora of mammals and pathogenic *E. coli* strains was not possible. *Clostridia*, which include human and animal pathogens, are anaerobic spore-forming firmicutes that produce noxious odours while fermenting proteins and lipids. The *Enterobacteriaceae* family within the Gammaproteobacteria includes the genus *Salmonella*, most of which are pathogens, and *Escherichia*, which also includes pathogenic strains [9].

Identification of bacteria was done by Gen III microplate BIOLOG microbial identification system. They were *Bacillus amyloliquefaciens, Bacillus ruris, Bacillus licheniformis, Bacillus subtilis* and *Kocuria varians.* Similar with He et. al. [10] that the *Bacillus* species almost appeared as majority in relation to the degradation of organic molecules.

IV. Conclusion

Bacteria were found in landfill soil of Jabor landfill were 19 different species of bacteria. Three Gram positive and 16 Gram negative bacteria. Bacteria were found in leachate tank were found 18 different species of bacteria. Five Gram positive and 13 Gram negative bacteria. They were *Bacillus amyloliquefaciens, Bacillus ruris, Bacillus licheniformis, Bacillus subtilis* and *Kocuria varians*. Future work may focus on application of consortium bacteria for municipal solid waste degradation.

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