

The Effect of pH and Aluminium to Bacteria Isolated from Aluminium Recycling Industry

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ABSTRACT

Aluminium recycling is one of currently growing industries in Indonesia. Abundant amounts of aluminium waste leads to the emergence of new industries. The waste produced from this kind of industry is not well processed yet. Bioremediation using isolated indigenous bacteria is one of the best options from treating the aluminium recycling wastewater. Since biological processes are closely related to the bacterial growth conditions, it is important to understand the effect of pH and aluminium exposure to bacteria. Six potential bacteria strains were obtained from isolation. *Vibrio alginolyticus* and *Brochothrix thermosphacta* were shown to be resistant to the aluminium exposure, as well as the acidic conditions. Both types of bacteria were able to survive on acid medium with pH 5. The higher the concentration of aluminium, the slower bacterial growth rate achieved. The MIC value of aluminium for *Vibrio alginolyticus* was 425 mg/l, *Brochothrix thermosphacta* was 325 mg/l and *Pseudomonas aeruginosa* was 200 mg/l.

Keywords: aluminium recycling industry; *Brochothrix thermosphacta*; indigenous bacteria; pH; *Pseudomonas aeruginosa*; *Vibrio alginolyticus*.

INTRODUCTION

The abundance of aluminium waste in Indonesia leads to the emergence of the aluminium recycling industry (BLH Jombang, 2016). Aluminium recycling is a series of processes to refine the aluminium waste into pure aluminium metal (Tsakiridis, 2012). Although these industries produce pure aluminium metal from waste, they still generate some impurities as by-product and wastewater from several processing stages (Ingustald, 2012). Further treatment of wastewater needs to be performed before discharge to water bodies in order to avoid environmental pollution.

Bioremediation is one of the most prospective types of treatment to be applied in recycling aluminium wastewater. It requires competitive capital cost as well as lower operation and maintenance cost compared to physicochemical treatment (Purwanti et al., 2017). In order to achieve the highest pollutant removal percentage, several

factors need to be concerned in applying the bioremediation technique (Deepali, 2011). Bacterial species, pH and pollutant concentration become the most important factors in bioremediation (Chau et al., 2014). Indigenous bacteria are known to have the best ability for metal bioremediation (Soumitra et al., 2012). The bacteria isolated from the metal contaminated environment tend to be more tolerant to metal and considered to be more effective for treating the metal pollution [Haytham 2016, Mats 2012].

The living environment for bacteria plays a role in bioremediation because of its correlation with the bacterial metabolism (Deepali, 2011). Although the use of indigenous bacteria is highly recommended, several factors such as pH and pollutant concentration need to be concerned. For instance, pH is highly correlated with bacterial enzymatic reaction and metal bioavailability (Chau et al., 2014). The pollutant concentration is related to the bacterial growth and the capability

to live in a contaminated medium (Khatun et al., 2012). To our knowledge, the effect of pH and aluminium on bacteria has not been widely studied yet. Understanding the effect of different pH and different concentration of aluminium exposure on the growth of isolated indigenous bacteria to achieve the optimum bacterial growth condition was the main objective of this research.

MATERIALS AND METHODS

Isolated bacteria

Three potential strains of bacteria, which were *Vibrio alginolyticus*, *Brochothrix thermosphacta* and *Pseudomonas aeruginosa* isolated from the aluminium recycling industry. These bacteria already pass the screening test and tend to have high resistance to aluminium. *Vibrio alginolyticus* is one type of aquatic bacteria that can be isolated from contaminated areas (Noguchi et al., 1987). *Brochothrix thermosphacta* is a type of bacteria that is widely found in plantation, agriculture and livestock. These bacteria were classified as superior because of their adaptability (Kilcher et al., 2010). *Pseudomonas aeruginosa* is a common type of bacteria that can be isolated from bodies of water (Hoiby et al., 2010). This bacterium is also known to be able to perform biosorption and bioaccumulation of aluminium inside its cells (Tuzen and Soylak, 2008).

Bacterial growth rate curve

The bacterial growth rate test was performed to determine the bacterial growth curve and to obtain the exponential growth time of each bacterial species. This exponential time will be used as a reference in the process of making bacterial inoculum with certain optical density (OD) in the next stage of research. The method used in the bacterial growth rate test referred to (Deepali, 2011). The bacterial growth rate test was done for 24 hours by shaking reactor at 150 rpm (Deepali, 2011). OD 600 nm observations were performed every 2 hours for 24 hours. The bacterial growth curve was made by plotting the tested OD vs time.

Effect of pH to bacterial growth rate test

The effect of pH test was done according to (Konishi et al., 1994), in order to measure the growth of bacteria under different pH conditions.

Aluminium exhibits amphoteric characteristic and has a good solubility in mainly acid pH. This test was performed for several pH, i.e. 3, 4, 5 and control medium (neutral pH = 8) to compare the growth of bacteria under normal and acidic conditions. This test was done by inoculating 2% of bacterial culture (Ibrahim, 2016) ($OD_{600} = 0.5$) (Purwanti et al., 2015) into pH adjusted nutrient broth medium (Merck, Germany). Adjustment of pH was done by adding HCl (Merck, Germany) into the Nutrient Broth Medium until the desired pH was achieved. The optical density 600 nm of bacterial cultures was observed every 2 hours for the first 24-hours, then checked daily until 72 hours of 150 rpm shaking (Purwanti et al., 2015) by using a spectrophotometer (Innova2000, Germany).

Effect of aluminium exposure to bacterial growth rate test

The effect of aluminium test was carried out to determine the ability of bacteria living inside aluminium-contaminated medium. The effect of aluminium test was tested by inoculating 2% of bacterial culture (Ibrahim, 2016) ($OD_{600} = 0.5$) (Purwanti et al., 2015) into several concentrations of sterile aluminium-contaminated nutrient medium, ranging from 0 to 1000 mg/l. The Aluminium-contaminated medium was made by mixing aluminium chloride solution and nutrient broth medium at certain ratio to achieve the desired concentration. Then, the reactors were shaken at 150 rpm for 72 hours (Purwanti et al., 2015). The optical density 600 nm of bacterial cultures was observed every 2 hours for the first 24- hours (or until the exponential growth was achieved), then checked daily until 72 hours of 150 rpm shaking (Purwanti et al., 2015) by using a spectrophotometer. The bacterial growth rate in an aluminium-contaminated medium was made by plotting OD checked vs time.

Minimum Inhibitory Concentration (MIC) of aluminium test

MIC in solid medium was obtained by streaking a bacterial culture onto the several concentrations of aluminium-contaminated nutrient agar surface, referring to (Mythili and Karthikeyan, 2011) and (Ruangpan, 2004). The aluminium-contaminated medium ranged from 0 to 1000 mg/l. The aluminium-contaminated medium was prepared by mixing 10 ml of sterile nutrient agar and 10 ml of sterile aluminium chloride (SAP, In-

onesia) solution inside a sterile petri dish. The streaked culture was then incubated at 37°C in an incubator (Memert+, Germany). The area of visual growth of 24 hours incubated culture was calculated and compared with the control medium. This test was conducted in two steps which were Range Finding Test (RFT) and Definitive Test (DT). All bacteria were tested in the same range for RFT. In DT, the bacteria were tested in a smaller range between the highest concentration of aluminium. MIC of aluminium for all bacteria was chosen from the highest concentration of aluminium that totally inhibits the bacterial growth (Ruangpan, 2004).

RESULTS AND DISCUSSION

Bacterial growth rate curve

All bacteria tested had the same exponential time of 6 hours. Several related studies have varied results for the exponential time of the bacteria tested. All tested cultures involved the bacterial species that have an exponential growth time of 4 to 8 hours [Ruangpan 2004, Tsakiridis 2012]. Half of the exponential time was used for initial shaking during the preparation of bacterial inoculums to be inoculated in the reactor. The bacterial growth for *Vibrio alginolyticus*, *Brochothrix thermosphacta* and *Pseudomonas aeruginosa* can be seen in Figure 1.

Effect of pH to bacterial growth rate

The higher OD obtained in lower pH, the more bacteria tend to be resistant to acidic condition (Ciric et al., 2010). On the basis of the re-

sults of pH test effect (Figure 2 to Figure 4), all bacteria showed a very low visual growth on pH 3 that indicated the inhibition of pH 3 to all bacterial metabolism. The highest OD was obtained by *Brochothrix thermosphacta*, with 1.856A in the end of the test period in pH 4 medium. The results obtained for *Brochothrix thermosphacta* were in accordance with (Leroi et al., 2012), which stated that this bacterium could grow well up to pH 4.8. *Vibrio alginolyticus* metabolism was totally inhibited by pH 3 and 4. Under pH 5 conditions, the growth rate of this bacteria was slower than under the normal conditions. Lower growth rate indicated that there were some enzymatic and metabolism reactions adjustment to adapt to the acidic conditions in medium (Fair and Larsen, 1981). Same results were achieved for *Pseudomonas aeruginosa*. This bacterium showed total inhibition in pH 3 and 4 medium. Reference (Farid and Larsen, 1981) and (Klein et al., 2009) stated that *Vibrio alginolyticus* and *Pseudomonas aeruginosa* can survive up to pH 5. On the basis of this result, *Brochothrix thermosphacta* exhibited the highest resistance to the acidic conditions.

Effect of aluminium exposure to bacterial growth rate

The higher concentration of aluminium, the lower the bacterial growth rate achieved (Maier, 2008). The results of aluminium exposure test were shown in Figures 5 to 7. On the basis of Figure 5, *Vibrio alginolyticus* can grow well in the aluminium-contaminated medium up to 1000 mg/l concentration, although lower growth and longer exponential time were observed at 500 and 1000 mg/l of aluminium concentration. *Brocho-*

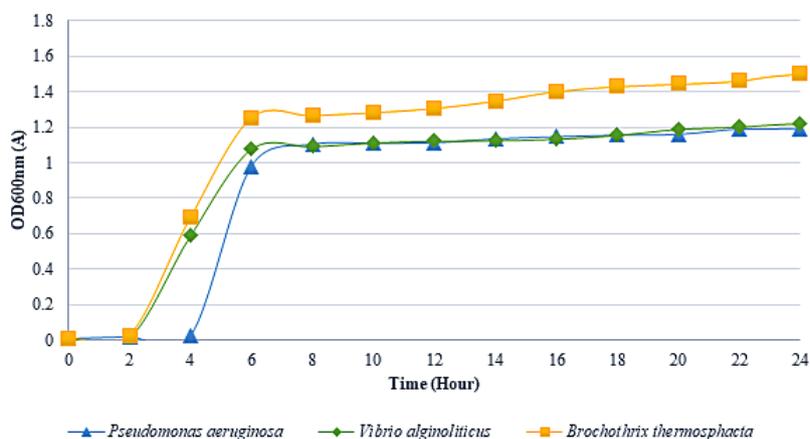


Figure 1. Bacterial growth

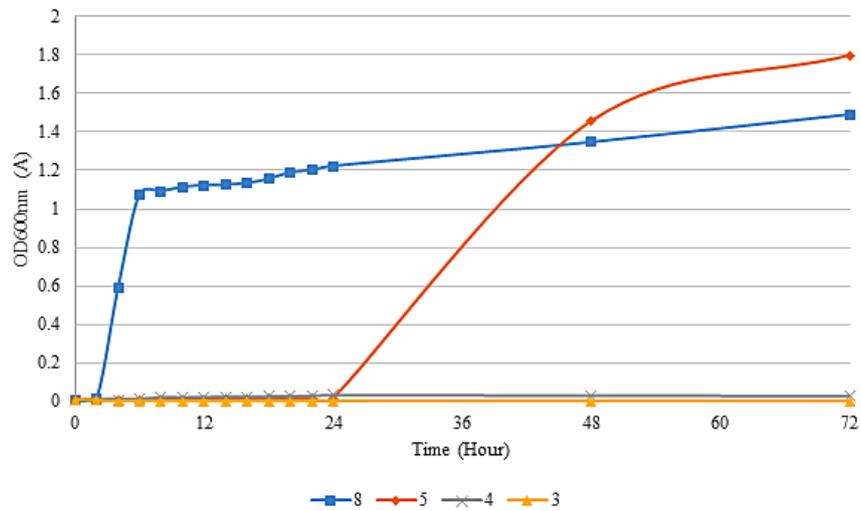


Figure 2. Growth of *Vibrio alginolyticus* in several pH conditions

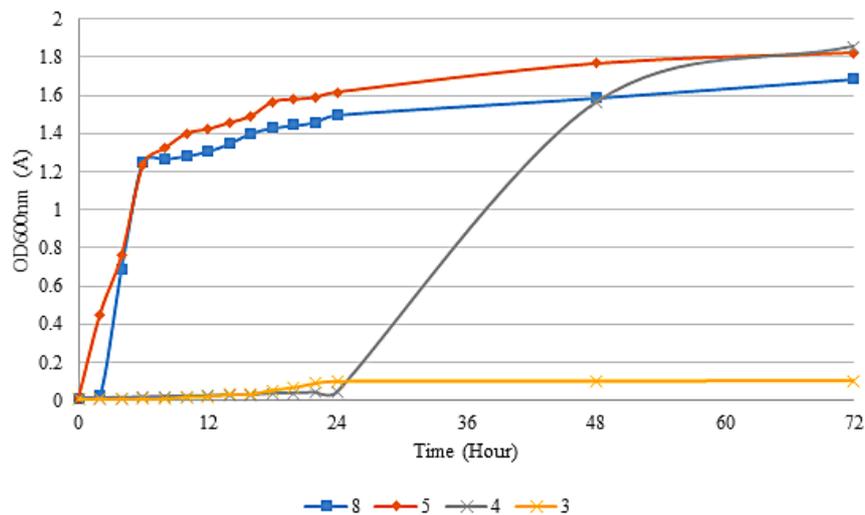


Figure 3. Growth of *Brochothrix thermosphacta* in several pH conditions

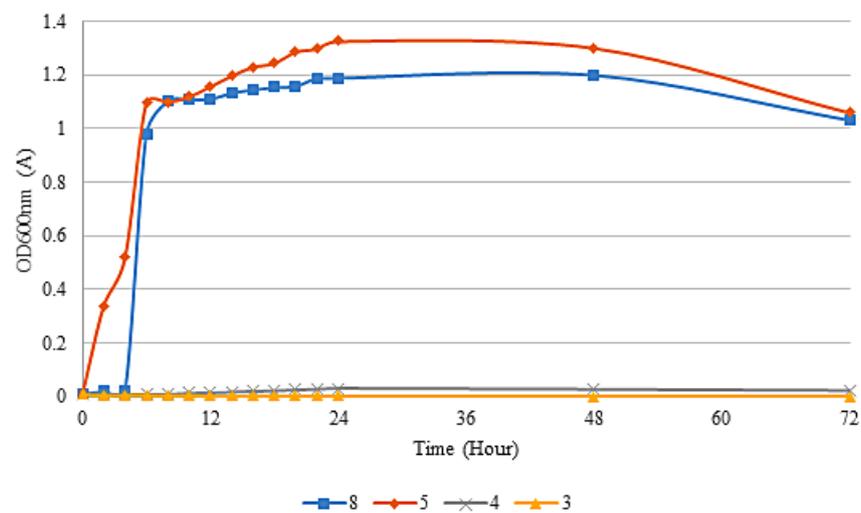


Figure 4. Growth of *Pseudomonas aeruginosa* in several pH conditions

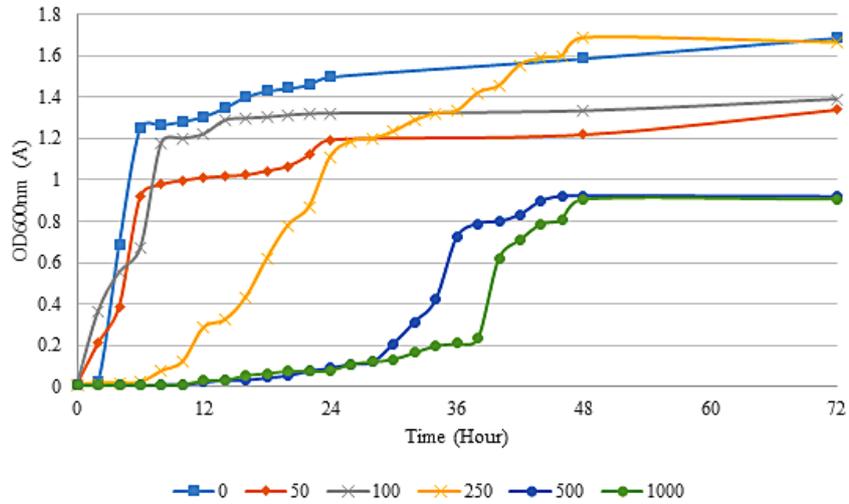


Figure 5. Growth of *Vibrio alginolyticus* in several concentrations of aluminium contaminated medium

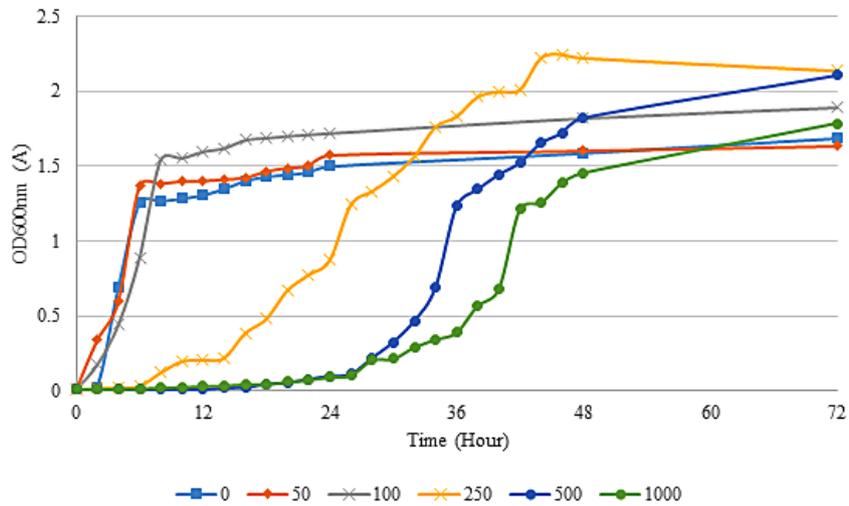


Figure 6. Growth of *Brochothrix thermosphacta* in several concentrations of aluminium contaminated medium

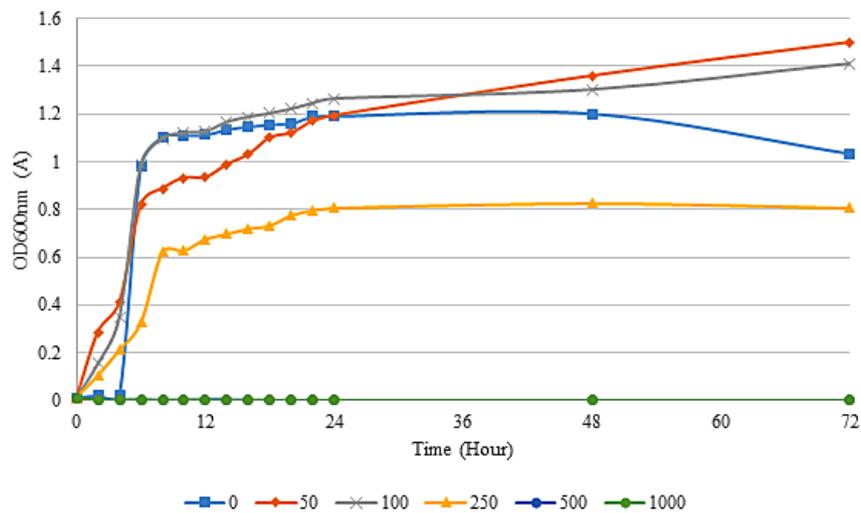


Figure 7. Growth of *Pseudomonas aeruginosa* in several concentrations of aluminium contaminated medium

thrix thermospacta showed good growth in the aluminium-contaminated medium up to 1000 mg/l concentration. On the basis of Figure 6, OD in all reactors were higher than 1.5 at the end of test period. Lower growth rate was observed in the reactor with 250 up to 1000 mg/l of aluminium concentration.

Pseudomonas aeruginosa had the lowest growth in the aluminium-contaminated medium. It showed a total inhibition in reactor with 500 and 1000 mg/l of aluminium concentration. Low growth was also observed in 250 mg/l of the aluminium-contaminated medium. Aluminium can decrease the growth rate of bacteria by adjusting or inhibiting some enzymatic reactions (Maier, 2008). Total growth inhibition by aluminium can also occur because of its binding with DNA, membranes and cell wall (Pina and Cervantes, 1996). Aluminium ion inside cell also caused competition of iron and magnesium uptake that could lead to a change in the enzymatic reaction (Pina and Cervantes, 1996). On the basis of the effect of aluminium exposure test, *Brochothrix thermospacta* showed the highest capability of living inside the aluminium-contaminated medium.

Minimum Inhibitory Concentration (MIC) of aluminium to bacteria

On the basis of Table 1, *Vibrio alginolyticus* showed the highest resistance to the presence of aluminium contamination on solid media, followed by *Brochothrix thermospacta* and *Pseudomonas aeruginosa*. *Vibrio alginolyticus* showed more than 100% visual growth up to a concentration of 250 mg/l aluminium. The lowest resistance was exhibited by *Pseudomonas aeruginosa* i.e. 0% visual growth at 250 mg/l aluminium concentration. The growth of bacteria compared to controls can exceed 100% due to different streaking on each petri dish. It can also be caused by the presence of complex enzymatic reactions related to

the utilization of substances present in the media (Maier, 2008). The results of RFT were shown in Figures 8 to 10.

On the basis of the results obtained from the RFT test, further analysis was performed to find more detailed concentrations that caused 100% of bacterial deaths. Further analysis was performed using a definitive (DT) test in the 250 – 500 mg/l concentration range for *Vibrio alginolyticus* and *Brochothrix thermospacta*, and 100 – 250 mg/l for *Pseudomonas aeruginosa*. The definitive test results (DT) can be seen in Figures 11 to 13.

The MIC values for all bacteria was chosen from the lowest aluminium concentration at which 100% death (no visual growth) occurred compared to the control ($p < 0.05$). The results of definitive test in Table 2 indicate that the MIC value of aluminium for *Vibrio alginolyticus* was 425 mg/l, *Brochothrix thermospacta* was 325 mg/l and *Pseudomonas aeruginosa* was 200 mg/l.

CONCLUSION

On the basis the observations, the acidic conditions can stunt the bacterial growth by adjusting its enzymatic reaction. The highest resistance to the acidic conditions was exhibited by *Brochothrix thermospacta* which can grow well up to pH 4. Increased aluminium concentration in medium can slow down and inhibit the bacterial growth. *Vibrio alginolyticus* and *Brochothrix thermospacta* showed good growth in the aluminium-contaminated medium up to 1000 mg/l concentration, although its growth rate was decreased along with the increasing of aluminium concentration. *Pseudomonas aeruginosa* growth was totally inhibited by aluminium starting from 500 mg/l concentration. The highest MIC value of aluminium was achieved by *Vibrio alginolyticus* with 425 mg/l while *Brochothrix thermospacta* was 325 mg/l and *Pseudomonas aeruginosa* was 200 mg/l.

Table 1. Surface growth percentage in range finding test

Aluminium concentration (mg/L)	Bacterial Growth Percentage (%)		
	<i>Vibrio alginolyticus</i>	<i>Brochothrix thermospacta</i>	<i>Pseudomonas aeruginosa</i>
0 (Control)	100	100	100
50	100	106	79
100	128	84	61
250	111	45	0
500	0	0	0
1000	0	0	0



Figure 8. Range finding test of *Vibrio alginolyticus*

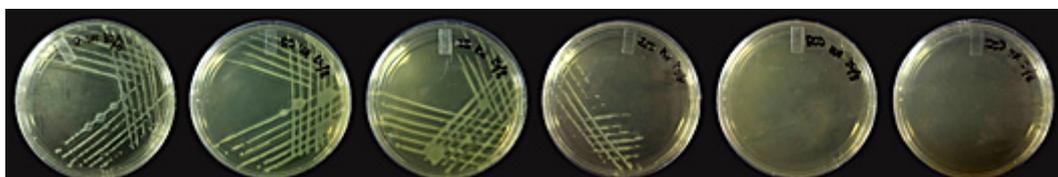


Figure 9. Range finding test of *Brochothrix thermosphacta*



Figure 10. Range finding test of *Pseudomonas aeruginosa*



Figure 11. Definitive test of *Vibrio alginolyticus*

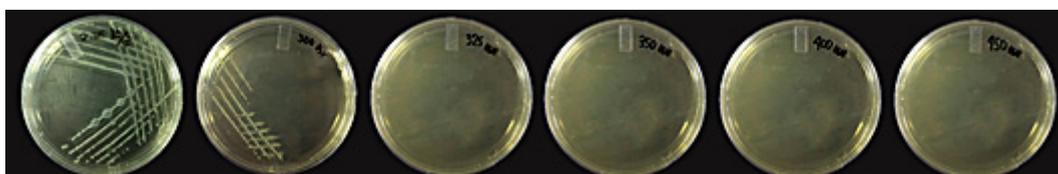


Figure 12. Definitive test of *Brochothrix thermosphacta*



Figure 13. Definitive test of *Pseudomonas aeruginosa*

Table 2. Surface growth percentage in definitive test

Aluminium concentration (mg/L)	Bacterial growth percentage (%)	Aluminium concentration (mg/L)	Bacterial growth percentage (%)	Aluminium concentration (mg/L)	Bacterial growth percentage (%)
<i>Vibrio alginolyticus</i>		<i>Brochothrix thermosphacta</i>		<i>Pseudomonas aeruginosa</i>	
300	79	300	20	125	46
350	64	325	0	150	31
400	43	350	0	175	18
425	0	400	0	200	0
450	0	450	0	225	0

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