Isolation and Characterization of Bacterial Community from Lake Water for Manganese Removal

Noraprilenna Jun[a], Fuzieah Subari[a]

[a]Faculty of Chemical Engineering, Universiti Teknologi MARA, Selangor, Malaysia

Abstract

Water scarcity was the result of imbalance natural phenomena across the world and attributed by the irresponsible act of human as well. A lot of cases reported worldwide on the contamination of heavy metal including manganese in water reservoir. Few methods applicable for manganese removal however biological method appear to be more promising than others. Biotic removal of soluble manganese (Mn^{2+}) to insoluble manganese (manganese oxide) by the microorganism known as manganese oxidizing bacteria (MOB). The MOB is phylogenetically diverse and can be isolated from various sources like lake, ocean and many other places. Hence, this study will be focusing on isolation and characterization of pure culture originated from surface lake water. The pure isolation technique will be conducted to isolate the community to its single colony. After being isolated, Gram Staining Test will be used to characterize the isolated bacteria based on its cell wall constituents and will be observed by using microscope. The manganese tolerance test conducted to test the ability of the bacteria to grow in the presence of manganese at various concentration. The number of colonies formed plotted into a graph versus manganese concentration after 24 hours incubation period. Bacterial growth was monitored for 24 hrs and the result were plotted accordingly. This study was successfully isolated 7 bacteria from the surface lake water. All of them were classified gram positive bacteria and were tolerance to the manganese at 100 mg/L.

Article Info

Article history:

Received date: 6th December 2019
Accepted date: 12th December 2019

Keywords:

Gram staining
Isolation
Lake water
Manganese
Manganese oxidizing bacteria

1.0 Introduction

The water scarcity around the globe become menace to human security due to the natural phenomenon and human activity causing the growing demand become more stressful condition to supply clean water or everyday uses [1]. These factors contributed to groundwater water contamination that also leads to many health adverse effect since it releases vast amount of heavy metals. One of it is manganese. According to United States Environmental Protection Agency, manganese in groundwater can cause three major problems namely as aesthetic effect, technical effect and also health problems [2]. In whatever way, it is impossible to have total avoidance power to the manganese in water ways since it is the fifth abundant metal on the surface of the earth [3]. The divalent soluble manganese (Mn^{2+}) can be oxidized to insoluble manganese oxides with the formula of MnO_x where X is a number in between 1 and 2. The manganese-oxidizing group is bacteria that were known has phylogenetically diverse and can be isolated from various origin like ocean, river and many more [4]. In this research project, the bacteria sample taken from the lake water. Compared to the other previous study, the difference is that most of other research used rDNA gene sequence but, in this research, the characterization only uses Gram Staining method. While the steps are less costly, but the precision might be less compared to the rDNA sequence method.

The purpose of this research is to study the characteristics of the single bacteria colony morphologically. The isolated bacteria will then undergo manganese tolerance test to examine the ability of the bacteria to grow in a various concentration of manganese.

2.0 Methodology

2.1 Material

The lake water was taken from the lake of Shah Alam, Malaysia. Nutrient Agar containing 25% Peptone, 15% meat extract and 60% Agar-agar

(GranuCult^{TM}, Merck Millipore, Germany) and Nutrient Broth containing 37.5% beef extract and 62.5% peptone (GranuCult^{TM}, Merck Millipore, Germany) used to supply the nutrient and also medium to grow.

2.2 Methods

Lake water was mixed in sterilized nutrient broth and incubated overnight. Pure isolation technique was used to isolate pure culture from surface lake water. The incubated mixed culture went through a serial dilution that is to dilute and reduce the bacteria concentration to a smaller amount so that the pure colony can form faster on the agar [5]. Each bottle containing 9 ml of distilled water was sterilized and added with 1 ml of incubated mixed culture labelled as S1. The S1 first must be swirl until homogenized to ensure well distribution of bacteria. 10 ml serial dilution will produce 10-fold dilution. The 7 bottles diluted to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}.

From the diluted solution, the last 4 dilutions (10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}) were taken to be spread on the fresh agar medium and replicated twice. A 0.1 ml of each dilution were dropped on the new nutrient agar medium. The hockey stick spreading the sample evenly or until the surface of the agar become slightly rough. The petri dish then sealed with parafilm, labelled and incubated for 24 hours at 37 ºC [6].

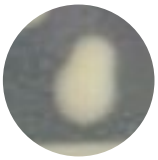
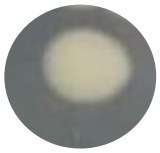
The incubated petri dish containing mixed culture was isolated into single colony by streaking method. The colony chose is based on different color, size and shape and was taken using the loop and streaked to produce new inoculum and single colony on the fresh agar medium. It was left in the incubator for another 24 hours.

The bacteria were classified into positive or negative gram groups through the Gram Staining Test after the single colony isolated. Gram-positive bacteria would retain the crystal violet's purple color while gram-negative bacteria would retain the safranin stain's pink color [7]. While for the Kinetic Growing Curve method[8], the inoculum was left overnight in 20 ml nutrient broth and mixed into 150 ml of nutrient broth the next day and the reading of optical density (OD) from the spectrophotometer (D2700, HACH, USA) were recorded every 1-hour interval. 1 ml of sample was drawn in centrifuge tube for cell dry weight (CDW) analysis. For CDW analysis, sample was centrifuge for 10,000 x g for 1 min. Supernatant was drawn and pellet was kept on the tube. It was left on the oven overnight and weighed afterwards.

If it is a positive-gram bacterium, the theory is that the manganese can be removed due to the cell structure of the positive-gram bacteria that can convert the soluble manganese into its insoluble form where black precipitate occurs. Manganese tolerance analysis were conducted on every species isolated on the new agar medium with 100 mg/L manganese concentrations. The plates will be incubated at 30 ºC for 24 hours. The colonies formed on the agar were counted for every species and the data was then tabulated on the result and discussion section.

3.0 Results and discussion

Table 1:Bacteria Characterization Based on Morphology [9]

Bacteria Morphology	**AF1**	**AF2**	**AF3**	**AF4**	**AF5**	**AF6**	**AF7**
Colony Shape							
Arrangement	Tetrad	Bud	Coccus Encapsulated	Coccus	Bud	Coccus	Coccus
Shape of the cell	Square of four irregular shape	Appendage	Spherical, concentrated at the centre with a shallow capsule	Spherical	Appendage with small parent like bud	Spherical	Spherical
Colony Margin	Undulate	Entire	Curled	Curled	Undulate	Entire	Entire
Colour	Creamy	White	White	Purple	Creamy	Creamy	White
Staining Colour	Purple	Purple	Purple	Pink Purple	Purple	Pink purple	Purple
Gram Staining	Positive	Positive	Positive	Positive	Positive	Positive	Positive

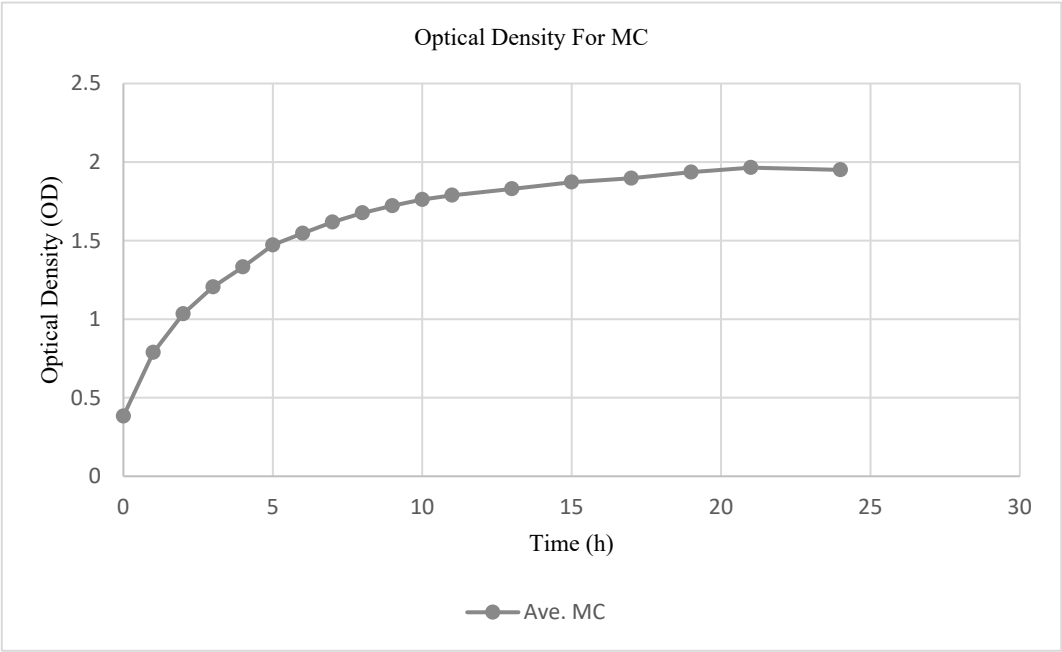


Figure 1: Growth curve of mixed culture

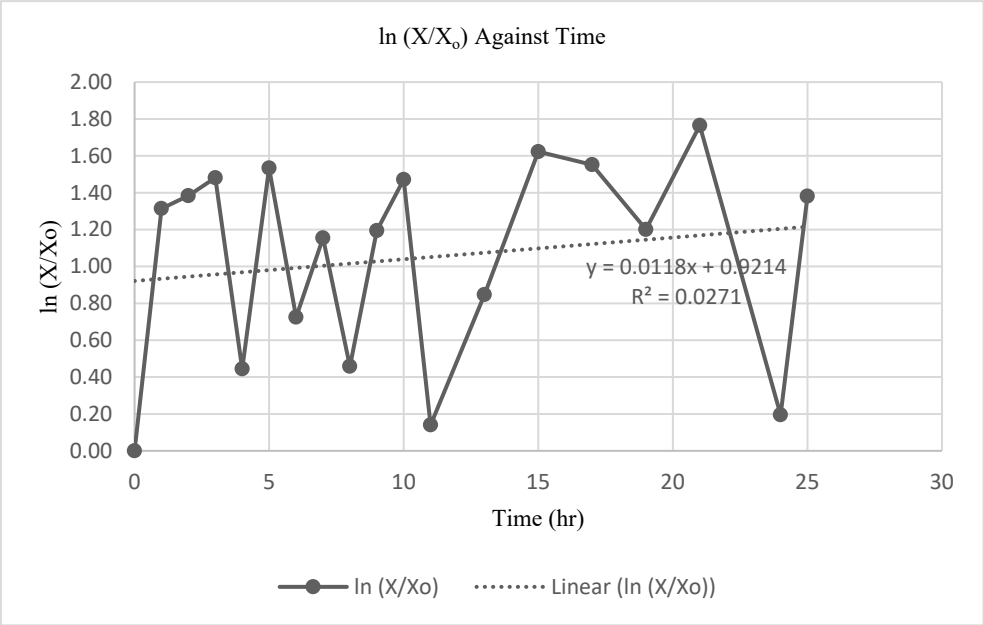


Figure 2: ln (X/Xo) Bacteria Mixed Culture Against Time

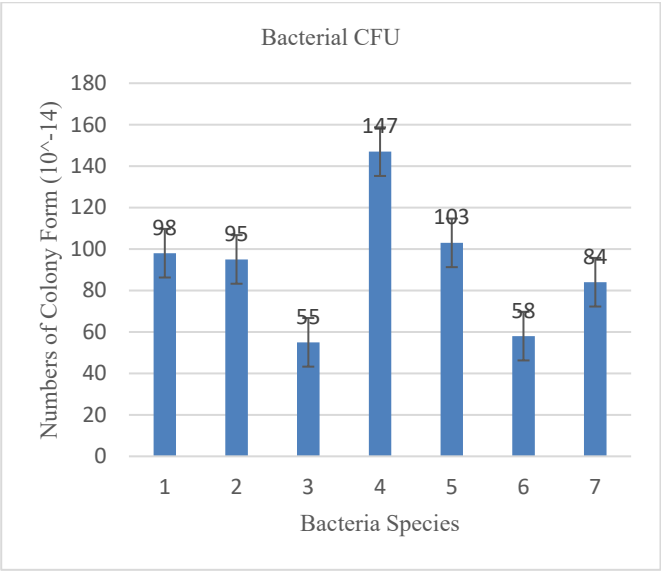


Figure 3: Bacterial Colony Forming Unit (CFU) against Time

From Table 1, the isolated bacteria formed 7 different single colony and classified into its own group according their colony shape, arrangement, shape of the cell, colour and their Gram Staining colour and group. All species indicate that they are the Gram-Positive category because, when examined under a light microscope after Gram staining at 100x magnifying, they have a distinctive purple appearance. Most of them appeared as coccus for the colony's form. Just two of them seemed to be budding. They may be coccus, but a slight protrudence can be seen on the surface of some of the cells (bud) during the observation.

Budding is a means of reproduction for some bacteria. This involves the formation of a bud on one end of the cell surface followed by genetic material replication. A duplicate of the substance joins the bud as it enlarges and eventually breaks off and splits from the bacterial cell of the parent[10]. While there is a range of white to cream for the color of the bacteria on the agar plate. But after streaking, only AF4 appeared as dark purple.

The kinetic growth curve for mixed culture was plotted as shown in Figure 1. For Mixed Cultured (MC) bacteria, the optical density indicates a positive growth trend as it obeys the increasing curve pattern of bacterial growth theoretically. Cellular activity, but not growth, characterizes this initial phase. A small group of cells are placed in a medium rich in nutrients which enables them to synthesize proteins and other molecules required for replication. Such cells are increasing in size, but in the phase, there is no cell division. But for MC, the lag phase cannot be defined as the curve has no distinctive bend to differentiate it between the lag phase and exponential phase. This is the moment when, after each generation time, the cells divide through binary fission and double in numbers. Metabolic activity is strong as it generates for division DNA, RNA, cell wall elements, and other growth-related substances.

However, for MC the exponential phase reaches up till 15 hours as the growing curve trend keep on arising.

During exponential phase, any treatment would be introduced as it multiplies actively. For example, antibiotics and disinfectants are most active in this growth phase as these substances usually target cell walls of bacteria or the processes of protein synthesis of transcription of DNA and translation of RNA. As this study conducted only for 24 hour and no sign of slowing down on growing for MC it might undergo the death phase after 36 hours to 72 hours.

Whereby for CDW as shown in Figure 2 indicates the Mixed Culture's growth curve over time. All samples collected in the dried Eppendorf tube each time OD was taken to determine the bacteria's Cell Dry Weight (CDW). CDW counts the sample's viable and dead cell. In this batch fermentation, the CDW reading should give a tally picture of the kinetic patterns of bacteria. For the lag phase of MC cannot be determined in MC, the weight of CDW on 0 hour is 0.01 g. The exponential cycle, as stated in OD, is up to 15 hours.

For the entire 15 hours, the graph becomes extremely unstable. For the first 1 to 3 hours, it develops smoothly and declines at 4 hours. The curve of OD and CDW were supposed to relate to each other. But the reading may differ due to the common error while the micropipette is being handled. Small changes of volume took using the micropipette can affect the final result of the graph. Or another factor, because there are different strains or forms of bacteria in the flask when one type of bacteria survives, the other type may be dead. Every consequent hour can be seen due to the extreme ups and downs pattern, the struggle to continue to multiply in a very small source of food causing the poor form to be suppressed.

The manganese tolerance test performed and the result depicts in Figure 3 where the number of colony formed in 100mg/L of manganese were counted. The result shows that if exposed to acute manganese concentration, all of the isolated organisms can withstand manganese at stated concentration. Of all isolated bacteria AF7 has the highest colony count that is 147 colonies while the lowest colony is observed for AF3, with only 56 colony formed

4.0 Conclusions

The research goal achieved as the result obtained confirmed the stated hypothesis. Seven different species were successfully isolated and developed by the bacteria. Because the isolated bacteria can be identified by their morphology, they can also be tested on the manganese tolerance test and produce readable amount of colony shaped on the Nutrient Agar medium even after overlay on the manganese. The research conducted may be useful in future research or may be implemented as one of the ways in which the bacteria are cultivated to treat the polluted water. It can also be used as an alternative way to treat other heavy metal in the wastewater treatment plant (WWTP) for industrial wastewater effluent.

Acknowledgment

I would like to thank Mrs Fuzieah Subari for being such a supportive hand in carrying out the research and clearing the way for me to understand better how to achieve the research goal. Special thanks to UiTM for supplying the laboratories and all the equipment and materials that are necessary to complete the work.

References

[1] B. Kifle Arsiso, G. Mengistu Tsidu, G. H. Stoffberg, and T. Tadesse, “Climate change and population growth impacts on surface water supply and demand of Addis Ababa, Ethiopia,” *Clim. Risk Manag.*, vol. 18, no. July, pp. 21–33, 2017.

[2] U. S. E. P. A. (EPA), “Secondary Drinking Water Standards_ Guidance for Nuisance Chemicals _ Drinking Water Contaminants – Standards and Regulations _ US EPA,” 2017.

[3] A. Gounot, “Microbial oxidation and reduction of manganese : Consequences in groundwater and applications,” vol. 14, pp. 339–349, 1994.

[4] Kenneth H. Nealson, *The Manganese-Oxidizing Bacteria*. Springer, New York, Ny, 2006.

[5] C. W. Brady, “Dilution Techniques and Calculations.” 2008.

[6] N. Rijal, “Spread Plate Technique_ Principle, Procedure and Results - Microbeonline.” 2017.

[7] F. Lowy, “Bacterial Classification , Structure and Function,” *Columbia Univ.*, pp. 1–6, 1884.

[8] S. S. M. and R. M. Punniavan Sakthiselvan, “Kinetic Studies on Cell Growth,” 2019.
[9] H. Ahern, “Microbiology: A Laboratory Experience.” 2018.
[10] J. T. Staley, P. Hirsch, and J. M. Schmidt, “Introduction to the Budding and / or Appendaged Bacteria c : £ : J,” 1981.
[11] M. D. Matovic, “Biomass Now: Sustainable Growth and Use.” 2013.