MALAYSIAN JOURNAL OF BIOCHEMISTRY & MOLECULAR BIOLOGY

The Official Publication of The Malaysian Society For Biochemistry & Molecular Biology (MSBMB)
http://mjbmb.org

KINETIC ANALYSIS ON THE EFFECTS OF LEAD (Pb) AND SILVER (Ag) ON WASTE CANOLA OIL (WCO) BIODEGRADATION BY SELECTED ANTARCTIC MICROBIAL CONSORTIUM

Khadijah Nabilah Mohd Zahri[1], Azham Zulkharnain[2], Salihu Ibrahim[1,3], Claudio Gomez-Fuentes[4], Suriana Sabri[5], Nancy Calisto-Ulloa[4] and Siti Aqlima Ahmad[1,6] *

[1]*Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2]*Department of Bioscience and Engineering, College of Systems Engineering and Science, Shibaura Institute Technology, 307 Fukasaku, Minuma-ku, Saitama, 337-8570, Japan.*
[3]*Centre for Biotechnology Research, Bayero University PMB 3011 Kano, Nigeria*
[4]*Department of Chemical Engineering, Universidad de Magallanes, Avda. Bulnes 01855, Punta Arenas, Región de Magallanes y Antártica Chilena, Chile*
[5]*Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[6]*National Antarctic Research Centre, B303 Level 3, Block B, IPS Building, Universiti Malaya, 50603 Kuala Lumpur, Malaysia*

*Corresponding author: aqlima@upm.edu.my

History
Received: 9 January 2020
Accepted: 2 April 2020

Keywords

Canola oil, heavy metal, degradation, Antractica, kinetic.

Abstract

Canola oil is used in most of Antarctic research station base and the possibility of discharging the waste canola oil (WCO) through the pipe in the kitchen is high, which leads to environmental pollution. Consortium bacteria form the Antarctic was isolated in degrading the WCO and tested regarding the degradation of oil with the presence of heavy metals. In this study, lead (Pb) and silver (Ag) were used to determine the behaviour of the bacteria consortium to degrade the WCO. The presence of lead allowed the degradation of oil to increased 48% to 56% while the availability of silver prevented the bacterial to grow and degrade the contaminants. Many types of data are best analysed by the fitting curve. The bacterial growth was fitted using both and linear and nonlinear regression curve where the exponential growth equation was used in a nonlinear curve. Bacterial growth with lead shown to be properly fit towards the curve with a high value of R^2 and low-value RMSE. In addition, there was no significant difference between linear and exponential regression curves for both conditions of the bacteria with heavy metals, lead and silver.

INTRODUCTION

The presence of heavy metals can severely affect the biological treatment through the bioremediation process using bacteria, which is one of the new techniques that appear to be the most cost-effective and beneficial on environmental contribution [1-3]. Low and high metal concentration can stimulate or inhibit the metabolism of the bacteria including in degrading pollutants. Metals could interact with the enzymes involved in biodegradation and general metabolism where it functions as enzymes cofactors [4-7].

Heavy metals are an increasing form of pollution in Antarctica caused by natural sources and anthropogenic activities from the research stations in the continents. The presence of heavy metals was discovered in Antarctica along with lead (Pb) and silver (Ag). These metals have high concentrations in Antarctica (McMurdo Station, Brazilian Antarctic Station, Coppermine Peninsular) with hydrocarbons contaminants along with other types of heavy metal such as barium (Ba), beryllium (Be), cadmium (Cd), Cobalt (Co) and Nickel (Ni) [8]. The availability of heavy metals in the environment could affect the growth and degradation of hydrocarbons-degrading bacterium including cooking oil since the biggest pollution in the Antarctic is hydrocarbons pollution.

Cooking oil (vegetable oil) is the most basic need for food preparation either in frying, grilling, baking or roasting [9]. In Antarctica, most of the research stations use canola oil as their oil since this unsaturated oil [10] has low freezing point, low cost and suitable for the use of various methods of cooking. However, by chance, waste canola oil (WCO) can cause pollution through the pipe into the

ecosystem and human activities in Antarctica are increasing from time to time for scientific research purposes [11], that already started on 1773 for the first expedition to the Antarctica by Captain James Cook for the development of Antarctic research until now [12]. Generally, the WCO is insoluble in polar substances and may lead to mechanical injury and toxicity to the living organisms besides the Antarctic biota that are sensitive to contaminants including penguins and seals [12].

Modelling kinetics is among the most advantageous techniques to study the performance of a biological process, wherein this study, the capabilities of the bacteria consortium able to degrade the contaminants and tolerate particular toxic metals namely Pb and Ag were determined. These two heavy-metal cations are considered as key factors of trace element in microbial chemical reactions that tend to bind to sulfhydryl (SH) group and inhibit the activity of sensitive enzymes [13]. Thus, this study aims to observe the effects on microbial growth kinetics with the presence of Pb and Ag in degrading WCO using a group of bacteria consortia directly from the Antarctic soils.

MATERIALS AND METHODS

Culture and media preparation

The soil sample (BS14) was collected from Base General Bernardo O'Higgins Riquelme, Antarctica and the soil sample were suspended in 10 mL of nutrient broth and shaken on orbital shaker 150 rpm at 10°C. The grown bacterium was subcultured twice to enrich bacterial growth. A modified minimal salt medium (MSM) was used as the medium for the bacterial, composing of 0.56 g/L KH_2PO_4, 4.74 g/L K_2HPO_4, 0.5 g/L $MgSO_4.7H_2O$, 0.5 g/L $(NH_4)_2SO_4$ and 0.1 $CaCl_2.2H_2O$ [14]. The medium was adjusted with HCl to pH 7.0 and autoclaved at 121°C for 20 min and 1% (v/v) of WCO was added after filtration of the oil with sterilised 0.25 µm filter syringe.

Effects of heavy metals on biodegradation and growth of suspension cells of Antarctic consortium bacteria

1 mL of the bacterial consortium was added in MSM from two days incubation samples of bacteria in nutrient broth. The Pb and Ag were diluted from 1000 to 100 part per million (ppm) in sterilised distilled water before being added with 1 ppm of diluted heavy metals in the MSM. The flasks were then incubated at 10°C and shaken at 150 rpm for 7 days with the control. Optical density (OD600) was measured using spectrophotometer every day for bacterial growth while oil reduction was measured by gravimetric analysis for degradation WCO. Residual WCO was measured using the solvents (n-hexane) to allow the separation of the two phases for the extraction of the oil from the media. The volume of extracted WCO was deducted from plate weight and the degradation percentage was calculated by considering the experimental control [15, 16].

Determination of growth curve model for BS14 microbial consortium on the presence of Pb and Ag

Kinetics models are convenient to obtain the data and understanding of microbial growth profiles using different mathematical models [17]. There are various types of growth curve model available to determine the intrinsic kinetic growth and for this study, the linear kinetics equation was utilised together with nonlinear regression through exponential kinetics equation using GraphPad Prism software (Version 5) and the data assessed the best model by measuring the goodness of fit for model [18]. This study generally aims at fitting the bacterial growth curve with linear and nonlinear exponential models, where the growth curves were drawn from predicted Equation 1 and 2, respectively, in a condition of unlimited threshold [19].

$$\frac{dN}{dt} = aN \quad (1)$$

$$N_t = N_0 e^{at} \quad (2)$$

Where Nt is the number of bacteria at time and a is the per-bacterium replication rate. Equation 2 can also be defined by Equation 3 as T is the doubling time, where T=In 2/r [20].

$$N_0 e^{at} = 2^{\frac{t}{T}} N_0 \quad (3)$$

These two approaches were used to understanding the growth during the cell cycle since the bacteria showed variations in cell growth patterns considering the time for them to grow.

RESULT AND DISCUSSION

Antarctic consortium BS14 was tested with the presence of Pb and Ag on the ability of the bacteria to degrade the WCO. According to **Figure 1**, the bacteria consortia were able to grow and resist in the presence of 1 ppm of Pb and showed higher degradation compared to the control (no heavy metals). Although Pb could destroy the proteins and inhibit enzyme actions [21], this bacterial community can resist them at 1 ppm in the presence of these metals and induced more the degradation of WCO. As stated by Jaroslawiecka and Piotrowska-Seget (2014), the high toxicity of Pb could evolve the mechanisms of many microorganisms that enable them to survive Pb exposure [22]. Some studies showed that *Escherichia coli* K-12, *Saccharomyces cerevisiae* and *Thiobacillus thiooxidans* can absorb, accumulate, or remove the Pb [23, 24]. This suggests that Pb has a less inhibitory effect on this bacteria community.

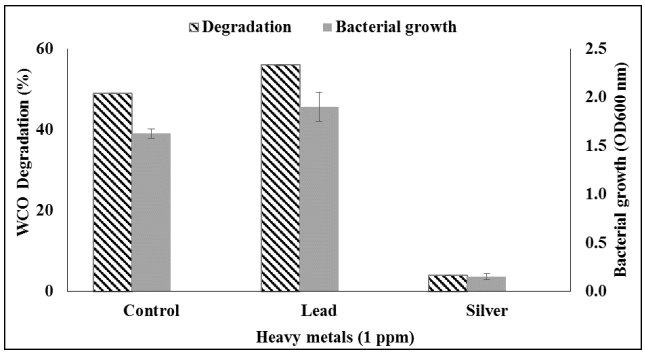

Figure 1. Growth and degradation effect of Pb and Ag (1 ppm) on the degradation of WCO by selected Antarctic microbial consortium for 7 days incubation period. Data represent mean ± SEM, n=3.

However, Ag was able to inhibit the growth of the bacteria and cause the degradation WCO by Antarctic consortium that cannot be degraded (**Figure 1**). Most of the bacteria died in the presence of silver due to the mechanism of antimicrobial action of the silver ion [25], where Jung et al. [26] discussed that silver ions caused marked inhibition of bacterial growth and were deposited in the vacuole and cell wall, which eventually suppressed the cell division and damage the cell structure. They also found out that the growth of *Staphylococcus aureus* and *Escherichia coli* rapidly dropped before 24 hours at 0.2 ppm of Ag at most concentrations. Meanwhile *P. aeruginosa* was unable to produce the desired enzymes (oxidase) in the presence of Ag [27] since silver

ion itself is a major mechanism of toxicity with thiol groups in membrane protein or enzymes [25].

Kinetics growth of Antarctic bacterial consortium on the presence of Pb and Ag.

Data from experimental value were fitted to linear and nonlinear regression of growth to provide a simple model. As shown from **Figure 2a**, the growth curve model for BS14 with the presence of Pb visually gave reasonably good fits of experimental data. The coefficients of determination (R^2) values were high at 0.8176. An R^2 value of 1 means a perfect fit and the higher the value, the better the fit [28]. For nonlinear regression, the line model was seen unable to generate the R^2 values due to the function of the value that compared the fits of the best-fit regression line only with a horizontal line [29]. However, the initial bacterial turbidity (Y^0) was at 0.8581 nm (**Figure 2b**).

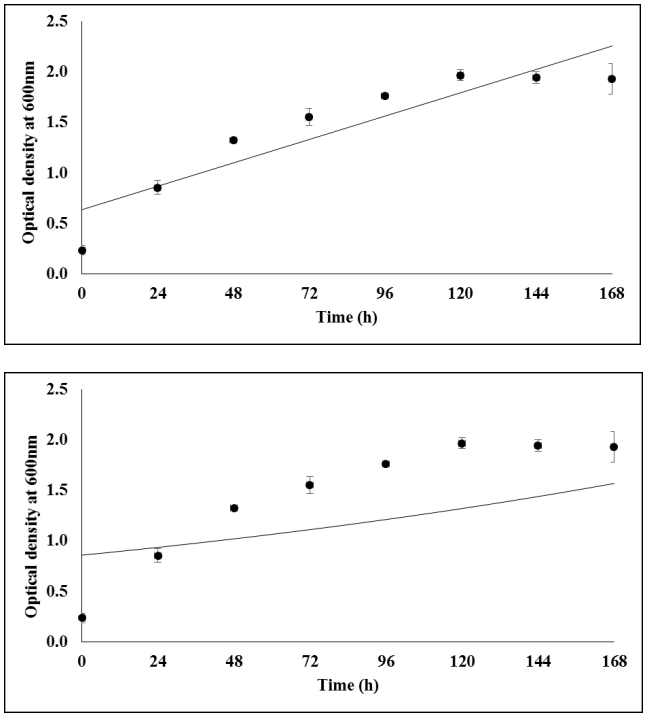

Figure 2. Linear regression growth curve (a) and exponential regression growth curve (b) on the effect of Pb on BS14.

Doubling time for the BS14 was determined through an exponential curve; 121 h was the time needed for the bacterial double in their size or number. These two models generated a square root of the variance of residuals (RSME) value, where the value was 0.265 and 0.332 for linear and exponential regression, respectively. RMSE should have a lower value that indicates a better fit [30]. This was shown through **Figure 2b**, in which the growth curve was lack of fit to the model of the exponential regression.

On the other hand, the kinetics growth curve for BS14 to degrade WCO with Ag were fitted properly (**Figure 3**) for both linear and nonlinear regression where the mean square error was 0.07431 and 0.07587 respectively. The graph was determined significantly as the value of p is less than 0.0001 although the value for R^2 square in this linear curve was at 0.5383.

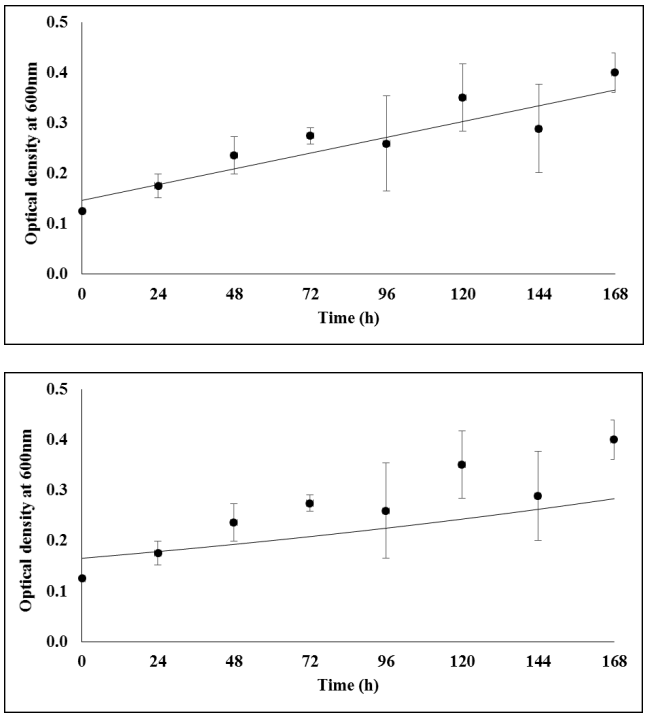

Figure 3. Linear regression growth curve (a) and exponential regression growth curve (b) on effect of Ag on BS14.

Since Y was the natural logarithm of the bacterial density at 600 nm, the Y^0 of the model was at 0.1655 nm and the doubling time for this growth curve was at 135.7 h (**Figure 3b**). The availability of Ag slowed the growth of the bacteria community as it ruptured the cells of the bacterial compared to the Y^0 value of the growth curve of Pb. All values for the growth rate (Y^0), time constant (Tau), rate constant (k) and doubling time fell within the 95% confidence interval of the distribution for both growth curves on the effects of Pb and Ag.

CONCLUSION

In summary, the kinetics studies on the effects of heavy metals on bacterial consortium growth in degrading WCO have been studied through linear and nonlinear methods' growth curve. Different types of heavy metals gave different effects on bacterial growth as some of them may act as essential nutrients and some are not. By using two different types of model, the linear regression growth curve suggested that the model have better performance to fit with the bacteria growth within 7 days.

ACKNOWLEDGEMENT

This work was supported by Universiti Putra Malaysia (Matching Grant PUTRA (9300436) and PUTRA Berimpak (9678900)), Yayasan Penyelidikan Antartika Sultan Mizan (YPASM) and Centro de Investigacion y Monitoreo Ambiental. The authors also would like to thank Chilean Army and the Antarctic General Bernardo O'Higgins Station staff especially the Chef; Suboficial Juan David Sandoval Navarrete and Sargento Juan Eduardo Cortínez Padovani, Instituto Antártico Chileno (INACH) and National Antarctic Research Centre (NARC).

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