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Determination of the Median Effective Concentration of Nano-Zero Valent Iron to the Marine Phytoplankton Species *Isochrysis galbana*

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ABSTRACT

Nano zero valent iron (nZVI) is often used in the remediation of organic and inorganic contaminant and has been hailed as an emerging remediation tool. Unfortunately, were few studies has been done on the toxicity of nZVI on organisms especially aquatic organisms. In this study, the toxicity of commercial forms (uncoated, organic coating, and iron oxide coating) of nano zero-valent iron (nZVI) to the marine phytoplankton *Isochrysis galbana* is revisited in terms of the determination of the EC_{50} or median effective concentration using nonlinear regression. Based on the four-parameter logistics equation, the median effective concentration for Nanofer 25S, Fe^{2+} and Fe^{3+} were 5.46 mg/L (95% confidence interval of 4.84 to 6.16), 70.89 mg/L (95% confidence interval of 37.26 to 134.9), 64.29 mg/L (95% confidence interval of 44.92 to 92.02). The results indicate that Nanofer 25S was the most toxic.

INTRODUCTION

Zero valent iron (ZVI or Fe^0) is a reducing agent that commonly used in remediating contaminant from environment. This reducing agent is readily available, inexpensive and can remediate various contaminants [1]. Furthermore, ZVI is proven to be effective in reducing stable contaminants such as chlorinated hydrocarbon from the environment [2]. This strong reducing agent is proven to be effective in reducing various types of metals from waste water such as chromium (Cr (VI)), copper (Cu), molybdenum (Mo) and lead (Pb) [3–5]. The utilization of ZVI is not only focusing on the remediating metals, it is also reported to be effectively in remediating leachate from agricultural activities. Nitrate contamination originating from pesticide and herbicide used in agriculture is reported to contaminate clean water source [6].

A combination technology of bacteria associated with ZVI is proven to be better in nitrate removal from agriculture waste water when compared to bacteria treatment and ZVI treatment alone [7,8]. ZVI also can be used in the treatment of pharmaceutical waste. Almost 90% of amoxicillin content can be removed by the ZVI from the respected waste water [9]. Whilst ZVI is commonly used in pollutant treatment, it is also can be used in industrial process. ZVI is reported to enhanced the delignification process of palm oil empty fruit bunch into a fine material that can be use later in biopolymer and biofuel industry [10]. This material also accelerate the synthesis of short chain fatty acid in the industry [11].

Despite of its beneficial property, ZVI treatment is also believed to contribute to the biota toxicity. The ZVI toxicity showed a vast effect towards wide range organism in the environment. This include effect towards microorganism, aquatic organism and plant [12]. Toxicity analysis towards phytoplankton population exposed to the ZVI was evaluated and the result shows a significant decreasing in the phytoplankton population [13]. Furthermore, utilization of ZVI in remediating pollution can effect microorganism population such as bacteria and archaea [14]. The contribution of ZVI toxicity is believed due to the formation of reactive oxygen species (ROS) formed through the Fenton reaction and Haber-Weiss reaction [15,16]. Other factor that contributes to ZVI toxicity is due to the accumulation of iron in the organism.

Accumulation of iron from ZVI contribute to malfunction in algae, plant and fish [17–22]. Lastly, accumulation of ZVI in environment reducing the dissolve oxygen (DO) amount in water body [20]. Due to the presence of toxicity characteristic in nZVI, it is important to determine the EC_{50} or median effective concentration by nZVI towards microorganisms. The median effective concentration can be calculated using mathematical software. An LD_{50} plot is in non-linear plot where discrimination between the value can only be calculated precisely through statistic [23]. This statistical approach is used to minimize the lack-of-fit error caused by the improper mathematical modelling selection [24].

MATERIALS AND METHODS

Acquisition of data

Data from the works of Keller et al. [13] from figure five showing the growth rate of I. galbana exposed to the various types of ZVI. The information was processed by the Webplotdigitizer 2.5 software that digitizes the scanned figure into comma separated values.

Four-Parameter Logistics Modelling

The four-parameter logistics equation was chosen in plotting a non-linear regression based on the least square fitting [25]. The equation is described as follows;

$$y = Bottom + \frac{(Top - Bottom)}{1 + 10^{(Log\ EC50 - x) * Hillslope}}$$

where y represent the growth rate (cell/H) obtained, x is the concentration of nZVI exposed to the phytoplankton (log unit), Log EC_{50} is the amount that produces 50% of signal response and Hillslope is the slope-like parameter (Hill coefficient). Multivariate analysis using the four-parameter supply model was calculated using the PRISM software (5.0) accessible from www.graphpad.com. Growth rate for *I. galbana* exposed to Fe^{2+} showed no inhibition trend and was excluded from the remodeling.

RESULTS AND DISCUSSION

The results of the modelling exercise show acceptable fitting of the data to the model (**Figs 1 to 3**). Based on the four-parameter logistics equation, the median effective concentration for Nanofer 25S, Fe^{2+} and Fe^{3+} were 5.46 mg/L (95% confidence interval of 4.84 to 6.16), 70.89 mg/L (95% confidence interval of 37.26 to 134.9), 64.29 mg/L (95% confidence interval of 44.92 to 92.02). The coefficient of determination or R^2 were 0.99, 0.78 and 0.88, respectively, which indicate that more data points are needed for the two latter cases to improve determination reliability. Despite this setback, the results indicate that Nanofer 25S was the most toxic. In comparison to other published works, the median effective concentration for zerovalent iron nanoparticles against cyanobacteria is 50 mg/L whilst for *D. magna* is greater than 1000 mg/L [26].

The determination of median effective concentration for toxicant along with its confidence interval is an important exercise neglected by numerous researchers. The uncertainty values obtained can be used to compare the toxicity of various toxicants and target organisms to each other. The least information that can be obtained is comparative assessment across publications. Although the comparison of various toxicants to the same organisms or the comparison of various organisms to the same toxicant is best done within the same lab and time, in reality this is impossible or uneconomical to be carried out.

If comparison is to be made across publications, the confidence interval should be used as an assessment of statistical significance, whether one result is more toxic or sensitive to another. Significant difference between two values occurs when the 95% confidence interval values are non-overlapped. However, when the 95% confidence interval values overlapped, this does not means signifcant or not significant at the $p<0.05$ level. What overlapped confidence interval values means is that more data and study are needed [27,28].

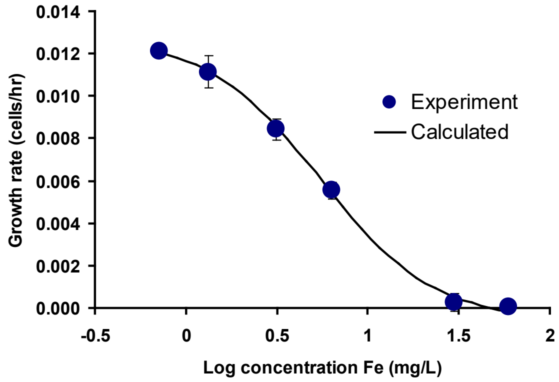

Fig. 1. The effect of Nanofer 25S on the growth rate for *I. galbana*.

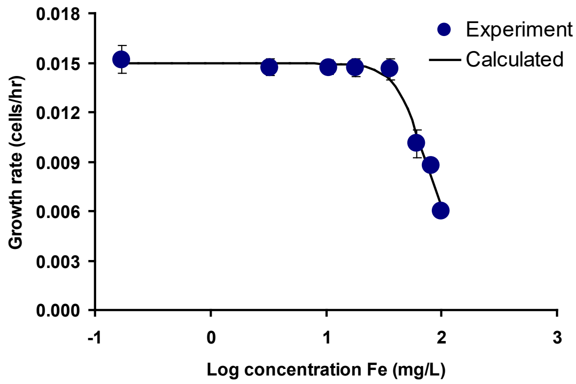

Fig. 2. The effect of Nanofer STAR on the growth rate for *I. galbana*.

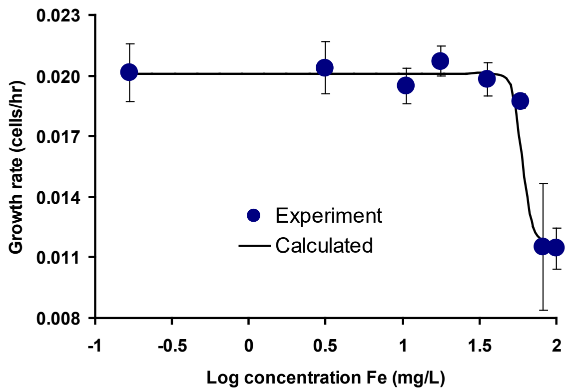

Fig. 3. The effect of Fe^{3+} on the growth rate for *I. galbana*.

CONCLUSION

Nano zero valent iron (nZVI) is an emerging remediation tool. Its toxicity to aquatic organisms, however needs to be determined through the use of nonlinear regression technique. In this study, the toxicity of commercial forms (uncoated, organic coating, and iron oxide coating) of nano zero-valent iron (nZVI) to the marine phytoplankton *Isochrysis galbana* is revisited in terms of the determination of the EC_{50} or median effective concentration using the four-parameter logistics model. Based on the four-parameter logistics model, the results indicate that Nanofer 25S was the most toxic.

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