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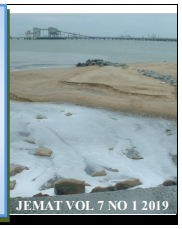

Mathematical Relationship of Optical Density, Total Viable Count and Microbial Biomass for Growth of *Serratia marcescens* Strain AQ07 on Cyanide

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ABSTRACT

This study illustrates the mathematical relationship between Optical Density (OD_{600nm}), Total Viable Cell Count (CFU/mL) and Microbial Biomass (mg/dry weight) of *Serratia marcescens* strain AQ07. The parameters were tested using spectrophotometric, colony-forming unit per ml and oven drying methods respectively. Bacterial density range of 0.2, 0.4, 0.6, 0.8 and 1.0 were correlated with the other two growth analyzing methods to correspond with 4.37 x 10^{15}, 1.02 x 10^{16}, 1.61 x 10^{16}, 2.19 x 10^{16} and 2.77 x 10^{16} CFU/ml and bacterial biomass corresponding to 1.01, 2.14, 3.29, 4.43 and 5.57 mg/ml respectively. This illustrates a clear relationship that can be explored in the batch growth remediation process so as to avert the continuous processes of viable cell count and microbial biomass techniques at every stage in the course of research. It saves cost, time and energy exhaustion as well help in simplifying research process since there is the need for researchers to report microbial parameter in mass (mg/ml) or Colony Forming Unit/mL rather than OD_{600nm}. Moreover; this technique can be utilized in modelling the bioremediation batch technique.

INTRODUCTION

Biological treatment using bacteria is a new technique that appears to be favorable as well as economically viable because of its benefit over the conventional process of cyanide treatment. Several bacteria have been reported to utilize various pathways in degrading cyanide [1]. It is considered to be a typical method that has been accepted overwhelmingly by the public and regulatory agencies because it can totally remove waste volumes and simply attain the lowest level of waste water effectively [2–4]. This technique could biodegrade cyanide devoid of creating a new waste like sludge or other by-products [5–7]. These advantages of the biological method make a more viable alternative [8–10]. Batch methods, utilizing cultures of bacteria are utilized for the biodegradation of cyanide in polluted effluents [11]. Modelling kinetics and assessment of biokinetic constant can be useful in understanding the performance of the biological method, the capabilities of bacteria for biodegradation, strategy, scaling up of the techniques in industrial scale [12–15]. In a batch reactor, in order to relate the pattern of substrate degradation with culture growth in the system, the kinetics of these two phenomenon should be analyzed [14].

The generally acceptable techniques for determining bacterial growth are Direct Cell Counts (CFU/mL), Optical Density (OD_{600nm}) and Dry Cell Weight (mg/ml) depending on the experimental conditions [12]. Since bacterial growth can be easily determined using Optical Density by spectrophotometric method, comparisons correlating OD_{600nm} and CFU/mL and Dry Cell Weight (mg/mL) can offer valuable information on biomass which is fundamentally important for modelling biodegradation process [12]. The aim of this work is to determine the mathematical relationship between OD_{600nm}, total viable cell count and dry cell weight of *Serratia marcescens* strain AQ07 used in the biodegradation of cyanide.

MATERIALS AND METHODS

Bacterial Strain

Serratia marcescens strain AQ07 has been established to biodegrade cyanide. It was isolated from non-cyanide polluted environment of Universiti Putra Malaysia (UPM) and registered in gene bank with accession number KP21391 [16]. It was tested to stand the toxicity of various heavy metals with the exception of mercury, which has a severe effect on it [17].

Analytical Technique

The density of the *Serratia marcescens* strain AQ07 was determined using Shimadzu U.V Mini 1240 spectrophotometer at λ_{600nm}. Phosphate Buffer medium was used as a medium for inoculum preparation.

Batch Flask Experiment

The experiments were conducted in 250 ml Schott bottles with a working volume of 100 mL, pH 6 inoculated with resting cells of the bacteria in an orbital shaker at room temperature for a period of 72 h. Range of bacterial density of 0.2, 0.4, 0.6, 0.8 and 1.0 are evaluated. The biomass tested for OD_{600nm} was centrifuged at 10, 000 rpm, 4°C for 10 minutes. The bacterial cells are suspended in a 100 mM phosphate buffer, washed twice and then serially diluted at 10^{-1}. Bacterial density was then measured by OD_{600nm} in each serially diluted suspension and was then inoculated on nutrient agar medium plates and incubated at 30 ˚C for 24 hrs to determine the total colony-forming unit per millilitre (CFU/ml). Duplicated cells of the same experiments using the same technique were used for measurement of biomass. This is done by centrifuging the serially diluted samples at 10, 000 × g, 4˚C for 10 minutes. The biomass pellets were collected and preserved in a hot air oven (50˚C) until constant dry weight DCW (mg/ml). All experimentations and measurements were carried out in triplicates, and arithmetic mean used all through the data analysis and calculations.

Regression analysis

Mathematical relationships between the cell concentration determined by different techniques; OD, CFU (cells/ml) and DCW (mg/ml) were assessed using linear least square techniques provided by statistical tool box of Microsoft excel 2010.

RESULTS AND DISCUSSION

Fig. 1 illustrates the positive linear relationship between CFU/ml and OD_{600nm} of measured data for *Serratia marcescens* strain AQ07, which is represented by the following linear polynomial equation:

$$y = 4E\text{-}17x \quad \text{.....Equation 1}$$

The analysis illustrates a very strong correlation with an R^2 coefficient of 0.9612. This indicates that OD_{600nm} of 1.0 approximately refers to 2.77×10^{16} CFU/ml. A similar correlation was conducted using *Bacillus sphaericus* HN1, illustrating an R^2 coefficient of 0.993 [12].

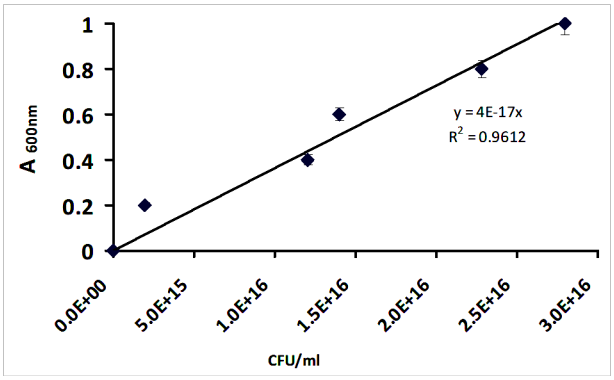

Fig. 1. Linear Positive Correlation between OD_{600nm} and Plate Count Technique (CFU/ml) of Measured Data for *Serratia marcescens* strain AQ07.

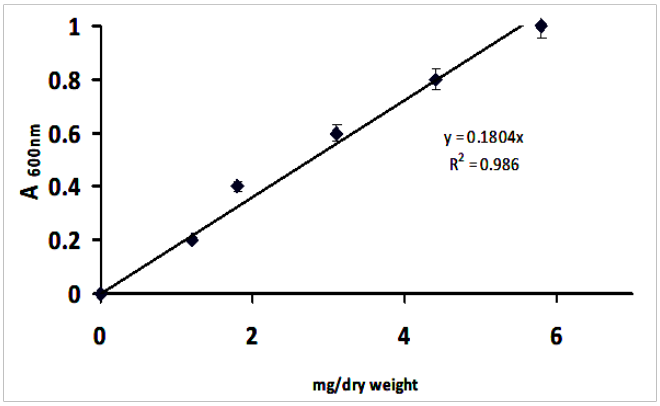

Fig. 2. Linear Positive Correlation between OD_{600nm} and Dry Cell Weight (mg/l) of Measured Data for *Serratia marcescens* strain AQ07.

Fig. 2 illustrates the linear positive mathematical correlation between DCW (g/L) and OD_{600nm}, which was also obtained for *Serratia marcescens* strain AQ07 and is represented by the following linear polynomial equation:

$$y = 0.1804x \quad \text{.....Equation 2}$$

The analysis illustrates a very strong correlation with an R^2 coefficient of 0.986. This indicates that 1.0 (OD_{600nm}) approximately refers to 5.567 mg/dry weight. Similarly, a correlation was carried out using *Bacillus sphaericus* HN1, which is represented by a linear polynomial equation with an R^2 value of 1, which represents 0.8819 g/L of biomass.

Table 1 illustrates the corresponding results of OD_{600nm}, Viable Cell Count (CFU/mL) and Bacterial Biomass (mg/mg). The correlation between optical densities of 0.2, 0.4, 0.6, 0.8 and 1.0 indicates equality with 4.37 x 10^{15} CFU/mL, 1.02 x 10^{16} CFU/mL, 1.61 x 10^{16} CFU/mL, 2.19 x 10^{16} CFU/mL and 2.77 x 10^{16} CFU/mL and bacterial weight of 1.01 mg/ml, 2.14 mg/ml, 3.29 mg/ml, 4.43 mg/ml and 5.57 mg/ml respectively. This correlation simplifies the determination of bacterial growth using the three methods simultaneously.

Table 1. Mathematical correlation between the three bacterial growth analyzing techniques.

S/No.	Optical Density (OD_{600nm})	Viable Cell Count (CFU/mL)	Bacterial Biomass (mg/mL)
1.	0.2	4.37×10^{15}	1.01
2.	0.4	1.02×10^{16}	2.14
3.	0.6	1.61×10^{16}	3.29
4.	0.8	2.19×10^{16}	4.43
5.	1.0	2.77×10^{16}	5.57

CONCLUSION

Establishing this type of mathematical correlations is very important in relating bacterial strains in batch processes of biodegrading substances such as cyanide which is used in gold extraction industries [18], bioremediation of oil from petroleum, hydrocarbon-contaminated water or soil and similarly in the use of bio-desulfurization of petroleum and its fractions [12,19–21]. It can accordingly simplify modelling, simulations and design of various bio-treatment methods using any bacterial strain. The limitation of this technique is it doesn't reflect the decline phase of the bacteria as conducting viable cell count at every stage of an experiment in the process of biodegradation.

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