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Mathematical Correlation between Microbial Biomass and Total Viable Count for Different Bacterial Strains used in Biotreatment of Oil Pollution

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This study illustrates mathematical correlations between viable cell count and biomass dry weight of four different bacterial strains, since microbial parameter often needed to be defined as mass (mg/ml) rather than colony forming unit CFU (cells/ml) or optical density OD in modeling of biotreatment batch process.

Key words: Mathematical correlation, total viable count, biomass, oil pollution.

Batch processes, employing bacterial cultures are in use for the degradation of polyaromatic compounds PACs, biotreatment of petroleum hydrocarbons polluted water or soil and biodesulfurization BDS of petroleum and its fractions,etc (Schirmer *et al*., 2000, Deriase *et al*., 2012, Deriase *et al*., 2013, El-Gendy *et al*., 2014).

Mathematical modeling, simulation and evaluation of bio-kinetic constants can be helpful for understanding the behavior of biological process, understanding the capacities of microorganisms for biotreatment, design and scaling up of the process for industrial scale. In a batch reactor, in order to relate pattern of substrate degradation with culture growth in the system, kinetics of these two phenomena should be analyzed (Nuhoglu and Yalcin, 2005).

The most widely available methods for measurements of bacterial growth are direct cell counts, measuring optical density using a spectrophotometer or dry cell weight, based on the experimental conditions.

Since the optical density OD of a bacterial solution can be easily determined using a commercially available spectrophotometer, equation relating OD to dry cell weight mg/ml or total viable count CFU cells/ml would provide useful information on biomass which is essentially useful for modeling biotreatment process.

This work aims to predict mathematical correlations expressing relationship between optical density, total viable count and dry cell weight of some bacterial strains used in different biotreatment applications in petroleum industry.

MATERIALS AND METHODS

Bacterial stranis

Bacillus sphaericus HN1 previously isolated for its ability to complete mineralize dibenzothiophene DBT through a Kodama pathway (Deriase et al., 2012).

Corynebacterium variabilis Sh42 previously isolated for its ability to degrade different polyaromatic compounds PACs (Deriase *et al*., 2013).

Rhodococcus erythropolis HN2 previously isolated for its ability to desulfurize DBT through the 4S-pathway (El-Gendy *et al*., 2014).

R. erythropolis strain IGTS8 has been recognized to desulfurize DBT via 4S-pathway (Kilbane, 1990).

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Analytical technique

Cell concentration was determined by measuring the OD using UV/Vis spectrophotometer (UNICAM, model 8625) at λ_{600nm}.

Medium

Luria Bertani LB medium is a complex medium used for inocula preparation and maintenance and was prepared according to Caro *et al.* (2008).

Batch experiments

Batch experiments were conducted in 1 l Erlenmeyer flasks, with working volume of 200 ml pH 7 LB, inoculated individually with bacterial strain (initial OD of 0.1), in an orbital shaking incubator set at 30°C and 150 rpm for a period of 48 h (the late exponential growth phase). The biomass was then harvested by centrifugation at 10,000 rpm, 4°C for 15 min and then re-suspended in fresh sterile saline (8.5 g NaCl/l distilled H_2O) and washed twice. The cells were then serially diluted 10^{-1}. Bacterial concentrations were then determined by measuring OD at λ_{600nm} in the serial dilutions of the suspensions and were also inoculated on LB-agar plates and incubated at 30°C for 48 h to enumerate the total colony forming unit TCFU (cells/ml). Duplicate cells of experiments with the same procedures were used for determination of biomass, by centrifugation of the serial dilutions at 10,000 rpm, 4°C for 15 min. Then the pellets of biomass were collected and kept in a hot air oven till constant dry weight DCW (mg/ml).

All experiments and measurements were done in four replicates and arithmetic averages were used throughout the data analysis and calculations.

Regression analysis

Mathematical correlations between cell concentration measured by different techniques; OD, CFU (cells/ml) and DCW (mg/ml) were estimated using linear and non-linear least square methods, applying Levenberg-Marquardt or Trust-Region algorithm, provided by statistics Toolbox with MATLAB software version 7.0.0.

RESULTS AND DISCUSSION

The predicted mathematical correlation between CFU/ml and OD_{600} of the measured data for *Bacillus sphaericus* HN1(Figure 1a) is represented by the following equation:

$$CFU/ml=4.25e12(OD)^{18.57} \quad ...(1)$$

Results indicate a strong correlation with $R^2 = 0.993$.

While a linear positive correlation between DCW (g/l) and OD_{600} was obtained for *B. sphaericus* HN1(Figure 1b) and is represented by the following linear polynomial equation ($R^2 = 1$):

$$DCW\ (g/l)=0.55147(OD)+0.33042 \quad ...(2)$$

Which indicates that 1.0 (OD) approximately refers to biomass of 0.8819 g/l.

By solving simultaneously equations 1 and 2, a non-linear mathematical relationship between CFU/ml and DCW g/l for *B. sphaericus* HN1 was derived (Figure 1c) and presented by the power equation:

$$DCW\ (g/l) = 0.55147\ [\frac{CFU/ml}{4.25e12}]^{0.0539} + 0.33042 \quad ...(3)$$

Also, a non-linear relationship between CFU/ml and OD_{600} for *Corynebacterium variabilis* Sh42 was predicted(Figure 2a) and can be represented by the following power equation ($R^2 = 1$):

$$CFU/ml=3.711e10\ (OD)^{16.8} \quad ...(4)$$

A linear positive mathematical correlation between DCW (g/l) and OD_{600} was also obtained for *C. variabilis* Sh42 (Figure 2b) and is represented by the following linear polynomial equation ($R^2 = 0.9801$):

$$DCW\ (g/l)=0.6333\ (OD)+0.1003 \quad ...(5)$$

Based on equations 4 and 5, a mathematical relationship between CFU/ml and DCW g/l for *C. variabilis* Sh42 was obtained (Figure 2c) and presented by the power equation:

$$DCW\ (g/l) = 0.6333\ [\frac{CFU/ml}{3.711e10}]^{0.0595} + 0.1003 \quad ...(6)$$

The predicted mathematical correlation between CFU/ml and OD_{600} of the measured data for *Rhodococcus erythropolis* HN2(Figure 3a) is represented by the following non-linear power equation ($R^2 = 0.9981$):

$$CFU/ml=5.78e8(OD)^{12.19} \quad ...(7)$$

This model equation is considered as a good correlation for calculating cell concentration CFU/ml as a function of OD, with goodness of fit represented by R^2=0.9981 (value closer to 1).

There was also, a predicted linear mathematical correlation between DCW (g/l) and OD_{600} for *R. erythropolis* HN2(Figure 3b) and is represented by the following equation (R^2 = 0.9935):

DCW (g/l)=0.0693(OD)-0.0187 ...(8)

Simultaneous solving of equations 7 and 8 correlates a mathematical relationship

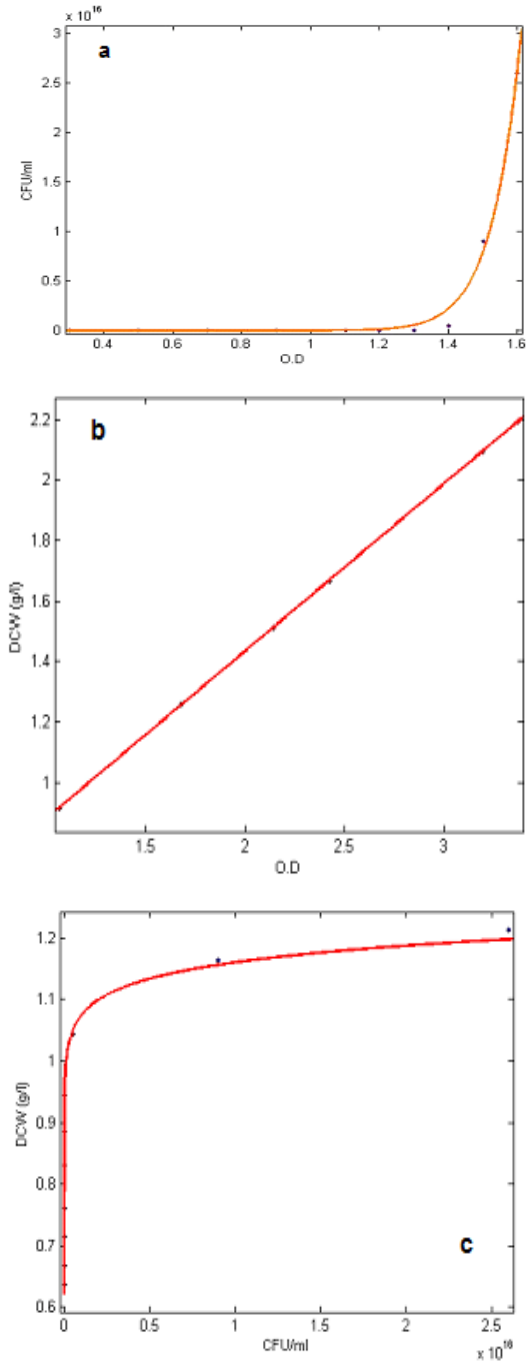

Fig. 1. Relationship between CFU, OD and DCW of *Bacillus sphaericus* HN1

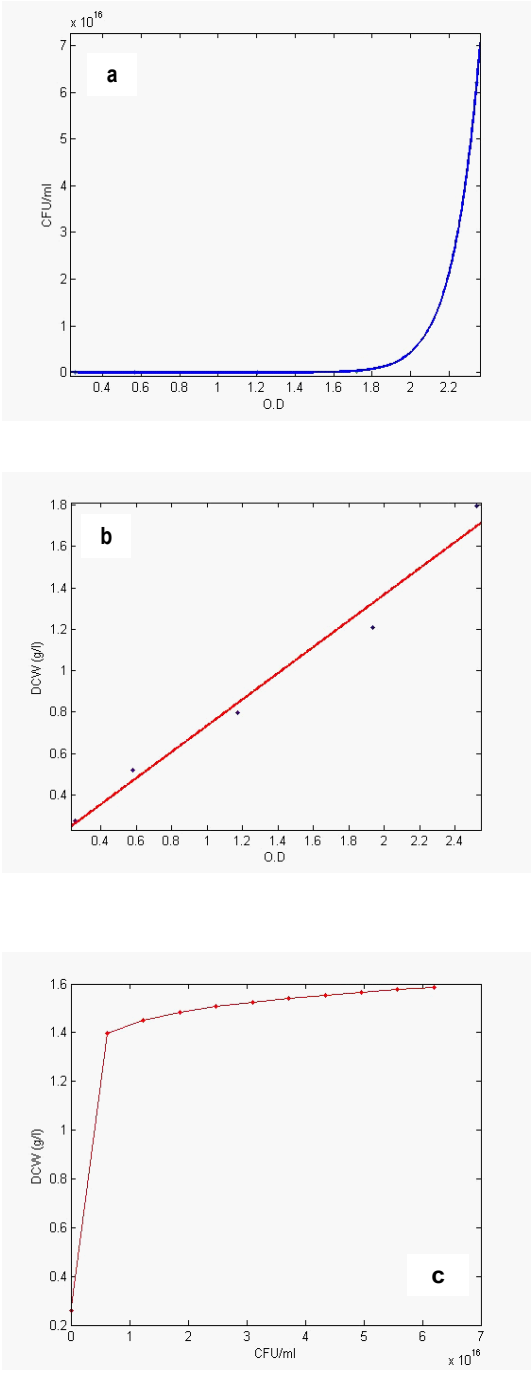

Fig. 2. Relationship between CFU, OD and DCW of *Corynebacterium variabilis* Sh42

between CFU/ml and DCW g/l for *R. erythropolis* HN2(Figure 3c) and can be presented by equation:

$$DCW\ (g/l) = 0.0693\,[\frac{CFU/ml}{5.78e8}]^{0.082} - 0.0187 \quad ..(9)$$

In case of *R. erythropolis* IGTS8, the predicted mathematical correlation between CFU/ml and OD_{600} (Figure 4a) is represented by the following equation ($R^2 = 0.9999$):

$$CFU/ml = 1.63e8(OD)^{5.854} \quad ...(10)$$

with a linear relationship between DCW (g/l) and OD_{600} (Figure 4b), presented by the following equation ($R^2 = 0.9947$):

$$DCW\ (g/l) = 0.08062(OD) - 0.0106 \quad ...(11)$$

Based on equations 10 and 11, a mathematical relationship between CFU/ml and DCW g/l for *R. erythropolis* IGTS8 was obtained

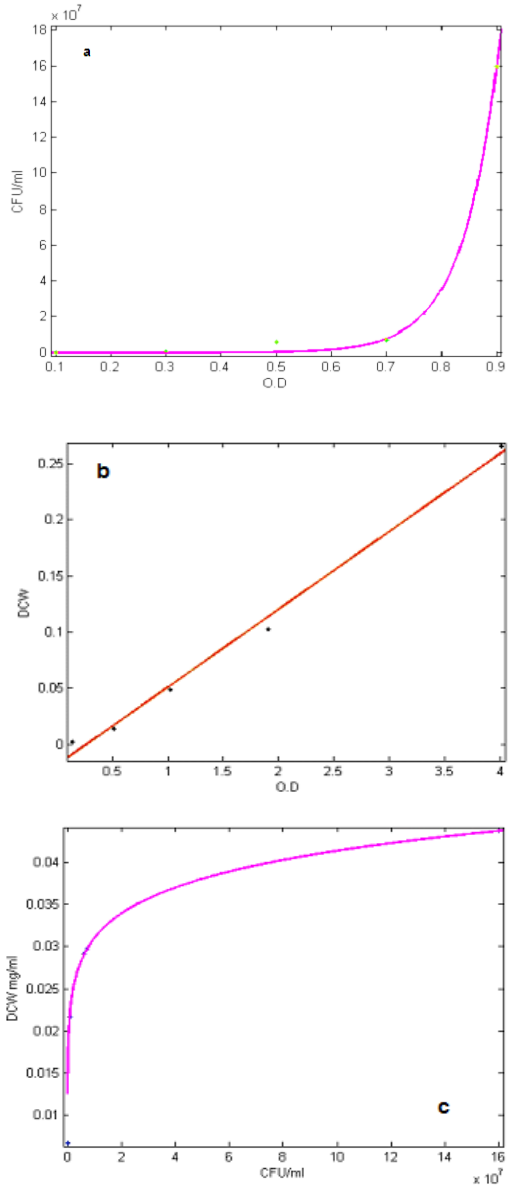

Fig. 3. Relationship between CFU, OD and DCW of *Rhodococcus erythropolis* HN2

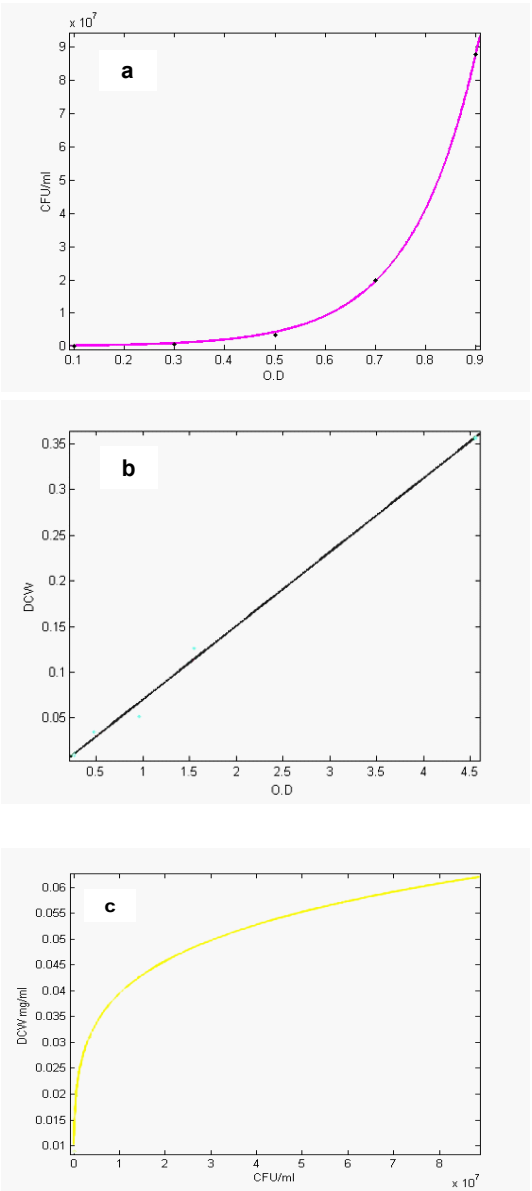

Fig. 4. Relationship between CFU, OD and DCW of *Rhodococcus erythropolis* IGTS8

(Figure 4c) and presented by equation:

$$DCW\ (g/l) = 0.0806\ [\frac{CFU/ml}{1.63e8}]^{0.1708} - 0.0106 \quad ...(12)$$

These correlations would be useful when applying these bacterial strains in batch processes of bioremediation of oil petroleum hydrocarbon polluted water or soil and also in application of biodesulfurization of petroleum and its fractions. This depends on how it would be easily determine the bacterial growth, by spectrophotometer, dry cell weight or plate counting methods. These correlations consequently, would facilitate modeling, simulation and design of different biotreatment processes using these bacterial strains.

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