2012 International Conference on Environment, Chemistry and Biology
IPCBEE vol.49 (2012) © (2012) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2012. V49. 37

Exposure Effect of Quantum dots on the Growth of E. coli Cells and Adsorption Kinetics on Growing Cells

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Abstract. Quantum dots (QDs) as semiconductor nanocrystals exhibit remarkable size-dependent optical properties, so they have a wide range of applications in different fields. Thus with the increasing production and application of QDs, the engineered QDs will possibly enter the environment, pollute environment and destroy ecosystem. Therefore, in this paper, the exposure effects of soluble CdSe QDs added into *E.coli* cells was studied, and results showed that in exponential phase, the specific growth rate of *E.coli* is $0.59h^{-1}$ and $0.66\ h^{-1}$ at QDs concentration of 200ppb and 90ppb respectively. Compared with the value of $0.71h^{-1}$ in control group (no QDs), QDs had an obviously inhibition effect on *E.coli* cells. In addition, the results form AFM showed the morphology change of *E.coli* cells before and after they were cultured in different concentration QDs solution. Furthermore, the further adsorption experiment showed that the adsorbed mass of QDs per unit mass of *E.coli* cells exhibits an exponential trend over culture time.

Keywords: quantum dots, *E.coli*, exposure effect

1. Introduction

Quantum dots (QDs), as nanocrystals, are a kind of semiconductors materials, which consist of a metalloid crystalline core and a "cap" or "shell" that shields the core. Newly synthesized QDs are given secondary coatings, such as protein and peptide, which improves water solubility and renders the QDs bioavailable[1].

Because QDs exhibit remarkable size-dependent optical properties[2-4], namely, the larger the dot, the redder (lower energy) its fluorescence spectrum. Conversely, smaller dots emit bluer (higher energy) light, they have a wide range of applications such as solar energy conversion[5], medical diagnostics[6], delivery agents[7] and others[8,9]. In addition, due to their bioavailable, a great deal of QDs are used in the field of biology[10,11].

Therefore, with the increasing production of QDs, the engineered QDs will possibly enter the environment, interacting with bacteria, and exerting an unknown effect on ecosystem. For CdSe quantum dots that are composed of heavy metal, it is suspected to be toxic if the bivalent Cadmium is released. John H.P et al. compared the effects to planktonic Pseudononas aeruginosa PG201 bacteria of ligand capped CdSe QDs and Cd(Ⅱ), and they found that QDs were more toxic to this opportunistic pathogen than cadmium ions[12]. In addition, Raphael S et al. also discussed the results of the exposure of bacteria to CdTe-core QDs and they concluded that QDs cytotoxicity is rather high due to the formation of TeO_2 and the possible existence of CdO formed by surface oxidation [13].

However, no data show the effects of soluble CdSe QDs on the growth of bacteria at different growth stages and thus this paper studied the exposure effects of soluble CdSe QDs added at different growth stages of E. coli cells to investigate the potential toxicity of QDs on ecosystems.

2. Materials and Methods

2.1. QDs and its Properties

QDs was purchased from Ocean Nano Tech, LLC from California of United States, and its properties was showed as Table 1.

Table 1 QDs and its properties

Supplier	Ocean Nano Tech, LLC
Catalog Number	QSH-620-04
Surface Group	Carboxylic acid
Solvent	Water
Emission Wavelength	620nm
Concentration	8μM
Particle Size	18.46nm
Zeta Potential	-26.17mv

2.2. *E.Coli* Cells

E. coli cells were purchase from Fisher Company and kept in refrigerator at 4℃, and cultured in standard Luria Bertani broth (LB) medium (catalog #:L3022, Sigma, US) at 37℃ overnight under aerobic condition.

2.3. Analysis Methods and Equipments

E.coli cells were cultured in incubator(New Brunswick Scintific). Optical density (OD) of the biomass in the culture medium was measured every hour at the wavelength of 600 nm with Spectrophotometer (Beckman, DU7700, US). SS/VSS (suspended solid/volatile suspended solid) was measured by weight method. Cd concentration was detected by ICP-MS (ICP-MS DRC Elan , PerkinElmer, US) followed the standard by ASTM[14]. Sample was centrifuged by centrifuge (Centrifuge 5418, Eppendorf). The morphology of *E.coli* was scanned by AFM (Agilent 5500, Agilent technologies).

2.4. Experimental Design

20 mL culture medium and 10 mL CdSe QDs were filled in 50 ml centrifuge tube (initial prepared QDs concentration is 90 ppb and 200 ppb as cadmium respectively). The tubes of the control group were filled with 20 ml culture medium and 10 ml DI water. OD standard curve (marked as standard) represents the cell growth under standard condition (The cells was inoculated in the freshly made culture medium without adding DI water or QDs solution. Initial inoculation of cells was around 3×10^7 CFU. The measured concentration of QDs was indicated by Cd concentration measured by ICP-MS.

2.5. Theory

For the exponential phase of cell growth, we can use the below equation to approximate the OD curve [14]:

$$X = X_0 e^{t\mu} \quad \text{(Eq.1)}$$

Boundary condition: $X = X_0$ at t=0. Where X is the mass concentration of cells in the liquid medium (proportional to OD value obtained by measuring the light absorbance at 600 nm); X_0 is the initial mass concentration of cells at the beginning of exponential phase; μ is the specific growth rate, h^{-1}; t is the culture time, h.

3. Results and Discussion

3.1. Exposure Effect on Cell Growth

At first, it is assumed that if the QDs have inhibition on *E.coli* cells, several possible phenomena are as follows: (1)The *E.coli* cells growth curve will be affected by QDs and manifested as a delayed lag phase or a decelerating exponential phase. (2) The morphology of *E.coli* cells will change. Next, the *E.coli* cells were cultured in different concentration QDs solution based on the experimental design in section 2.4, and the results shows that compared to standard curve of growth curve (defined in Section 2.4), the experimental groups had obvious drop at each sampling time in terms of OD growth and the growth inhibition comes from two major mechanisms: medium dilution effect and QD exposure (Fig.1). However, compared to the control groups(defined in Section 2.4), the experimental groups had no lag at each sampling time in terms of OD growth, even it can not find any QD exposure effect. Which may derive from OD value represents the results of microbial population growth.

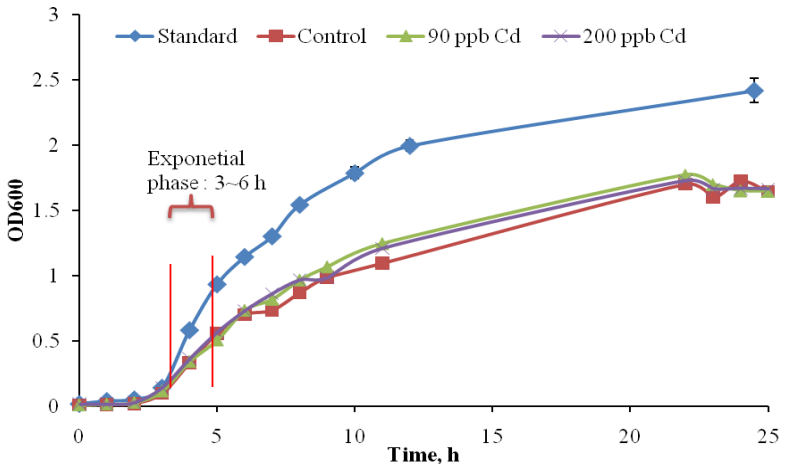

Fig. 1: Comparison of OD curves under standard, control, and two addition levels of QDs

Therefore, we selected the exponential phase as studied object, and based on the above mentioned kinetic equation(Eq.1), the OD data within the exponential phase was fitted and fit parameter returned the specific growth rate (μ) and other fit parameters are shown in the below Table 2. It can see that the standard curve μ has the highest valus than the other groups. The decrease in control group compared to the standard roughly represents the dilution effect of medium on cell growth. With addition of QD, μ decreased as QD concentration increased, indicating that QD has an inhibition effect on the cell growth.

Table 2 The fit parameters based on Eq.1

	Fit parameter(μ,h^{-1})	OF	R^2
Standard	0.76	0.27	0.80
Control	0.71	0.23	0.85
90ppb	0.66	0.18	0.93
200ppb	0.59	0.17	0.90

In addition, AFM was used to record the morphology change of *E.Coli* cells before and after it was cultured in 200ppb QDs solution and the Fig.2 shows the morphology before they are cultured, and compared with Fig.3, it can find that *E.coli* cells are slimmer and slimmer after they are cultured in 200ppb QDs solution for 25h, which also may result from QDs exposure effect.

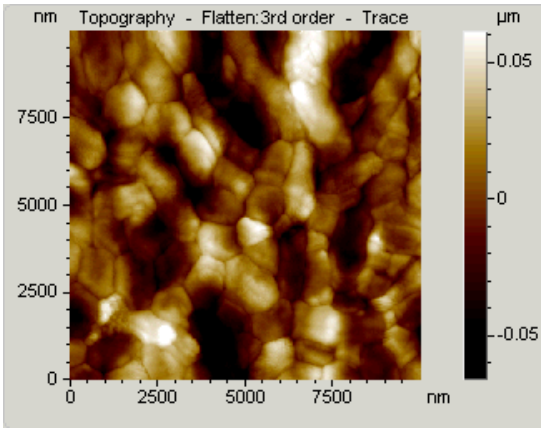

Fig.2: AFM figure before cultured in 200ppb QDs solution

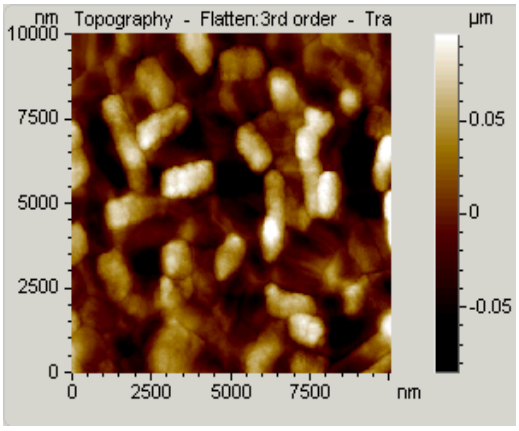

Fig.3: AFM figure after cultured in 200ppb QDs solution for 25hours

3.2. Adsorption of QDs on the growing *E. coli* cells

In order to investigate QDs exposure effect to *E.coli* cells, the following hypothesis is put forward, namely if the core of QDs is exposed to *E.coli* cells, QDs should firstly is adsorbed on the surface of *E.coli* cells and then the cores of QDs are exposed. Therefore, further hypothesis is that whether there exists a constant, which is similar with concentration coefficient, in terms of the adsorption of *E.coli* to QDs, and it can describe the adsorption quantity of QDs by *E.coli* cells. So in different culture time, QDs concentration was measured by ICP-MS, and the results was showed in Fig.4. From which, it can show the QD

concentration in the supernatant expressed as Cd concentration and the adsorbed mass of QDs per unit mass of *E.coli* cells. For both additions of 90 ppb and 200 ppb levels of QDs, Cd concentrations have a similar trend with culture time. Within the first 2 hours of addition, QD concentrations decreased from 90 ppb to 50 ppb and 200 ppb to 143 ppb and afterwards the QD concentrations for 200 ppb level of QDs had obvious fluctuations over culture time while for 90 ppb level of QDs the concentration was stable at around 50 ppb. Fig.4 also shows the adsorbed mass of QDs per unit mass of *E.coli* cells which exhibits an exponential trend over culture time for both addition levels of QDs. This is probably because the addition of QDs was constant, but the cells were growing which results in a decreasing ratio of adsorbed QDs/VSS.

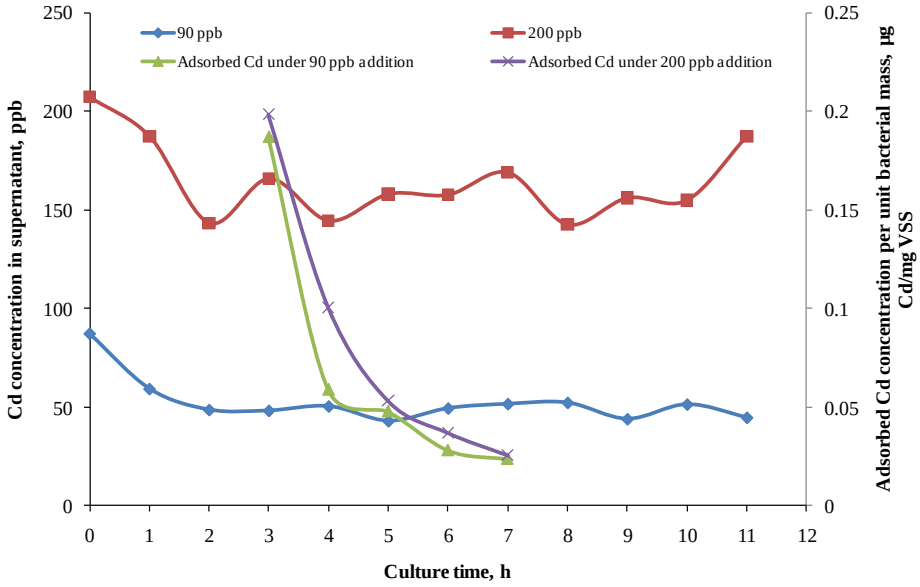

Fig.4: QD concentration in the supernatant and the adsorbed mass of QDs per unit mass of *E. coli* cells

4. Conclusions

(1) The *E.coli* growth curve was not lagged, but with addition of QD, μ decreased as QD concentration increased, indicating that QD has an inhibition effect on the cell growth.

(2) The adsorbed mass of QDs per unit mass of *E.coli* cells exhibits an exponential trend over culture time for both addition levels of QDs, which is probably because the addition of QDs was constant, but the cells were growing which results in a decreasing ratio of adsorbed QDs/VSS.

5. Acknowledgements

Assistant professor Yongsheng Chen and Wen Zhang from Georgia Institute of Technology are gratefully acknowledged for their very helpful suggestions and support.

6. References

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