DECOMPOSITION OF CdSe QUANTUM DOTS BY

*E. coli* AND AN ANTARCTIC BACTERIUM

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Masters of Science

by

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DECOMPOSITION OF CdSe QUANTUM DOTS BY

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DEDICATION

This written document symbolizes my enthusiasm to pursue education to this level and is dedicated to my beloved parents who encouraged and inspired me at the beginning and to my dear teacher, Dr. Thomas G. Chasteen, for his patience, great understanding and memorable teaching at the end.
ABSTRACT

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There were three phases in this research work. In the first phase, in general, the research time and work were dedicated to searching for viable quantum dot-tolerant bacteria, growing and storing metalloid-resistant bacteria, and studying CdSe quantum dot synthetic techniques in which incubation times and temperature of the synthetic processes were examined.

In the second phase of research, time and work were committed to bacterial growth curve experiments in which we found: 1) that a bacterium isolated from Antarctica, A0LBCd, had a higher specific growth rate in aerobic conditions than wild-type *E. coli*, BW 25113; and 2) that A0LBCd and BW could tolerate and grow well with glutathione-capped CdSe quantum dot suspensions in LB medium as high as 1500 ppm with little effect upon specific growth rates, and fluorescence studies in which we found that glutathione-capped cadmium selenide quantum dots exhibited relatively weak resonance Rayleigh and nonlinear scattering in sterile LB medium as well as strong fluorescence when exposed to UV light.

In the final phase of this research, much time and work was dedicated to analyses by gas chromatography with fluorine-induce chemiluminescence or mass spectrometric detection of headspace samples of bacteria exposed to *unwashed or washed* glutathione-capped quantum dots and to the investigation of organo-selenium volatile components in the bacterial headspace. The source of organo-selenium compounds in cultures growing in the presence of glutathione-capped CdSe quantum dots was determined to be selenium on
the surface of those unwashed nanoparticles, left over from the synthetic process, not from
the bacterial degradation of those quantum dots.

KEY WORDS: Quantum dots, Nanoparticles, Hydrothermal synthesis, Microwave
irradiation, Fluorescence spectroscopic study, Nanoparticle degradation, Rayleigh
resonance scattering, Non-linear resonance scattering, Antarctic bacteria.
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My special acknowledgement to Dr. Donovan C. Haines not only for giving me permission to use the Varioskan instrument and centrifuges but also for his support and teaching which enabled me to perform systematic experiments with a great saving of my time. And also, I am thankful to his guidance for my research and at writing as well.

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Finally I want to thank especially my family members, who supported me in the absence of my active part as husband and father—my wife and for my sons, and most of all, my three-year-old one.
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CHAPTER I
INTRODUCTION

Wild Type *E. coli* and Antarctic Bacteria

*Earth’s Early Inhabitants and Ancestors to Modern-Day Bacteria*

Many of us believe bacteria are harmful and troubling creatures because they are very much known to us with association of major diseases such as cholera, pneumonia, and tuberculosis. They are also associated with uncomfortable infections: whooping cough and chicken pox and, in addition, usual inconveniences like food spoilage. After the invention of the microscope, those tiny creatures were made visible to scientists, who were able to subsequently treat the causes of devastating epidemics (Tortora et al. 2010). Now we know some bacteria are harmful (pathogenic), but there are many not harmful but useful kinds. The nitrogen redox cycle is one of the most noteworthy elemental cycles in the biosphere where bacteria are involved in nitrogen fixation, a redox process that provides nitrogen (in usable form) for amino acid and nucleic acid synthesis (Gest 2003). *Acidithiobacillus ferroxidans* (metal oxidizing chemolithiotrophic bacteria) are employed in the metal sulfides industry to facilitate the metal solubilization process (Madigan et al. 2012).

The earth was anoxic—little or no molecular oxygen—during the first 1.8 billion years (Madigan et al. 2012); molecular oxygen began to accumulate in the atmosphere as a result of the oxygenic photosynthesis by cyanobacteria which had evolved from the anaerobic green and purple bacteria—photosynthetic bacteria. Biologists believe the earliest known bacteria, autotrophs, also known as hyper-thermophiles, ancestors to modern day bacteria, could have used CO₂ (as carbon-source) and H₂ (as fuel source) in
their primitive cells at the early metabolism stages (Madigan et al. 2012). These scientific findings lead to the famous Gaia Hypothesis which describes how the earth became alive—and the earth would not possibly be as much alive without bacteria (Lovelock and Margulis 1974).

**Aerobic and Anaerobic Bacteria**

Bacteria are single-cell organisms that reproduce by fission and must absorb their nutrients by diffusion. Aerobic bacteria need molecular oxygen to live and produce energy from nutrients. However, many aerobic bacteria can live in the absence of oxygen when they are subjected to anaerobic conditions. These microbes are called facultative anaerobes. *Escherichia coli* (*E. coli*) is an example of these organisms (Gest 2003).

A wild type *E. coli* strain and A0LBCd, a naturally occurring cadmium-resistant bacterial strain isolated from samples collected in Antarctica, were used in this research to investigate whether bacteria can biodegrade CdSe quantum dots.

**Decomposition of Quantum Dots (QDs)**

**Quantum Dots Stability**

Quantum dots—semiconductor nanocrystals—can be seen in a number of applications including optoelectronics (QD LEDs), solar cells, biological imaging tools (QD labels and sensors) and nano-drugs (QD delivery vehicles); therefore, it is important to consider the stability of quantum dots or, in another term, the ability to withstand decomposition in such applications (Rogach 2000; Karan and Mallik 2007; Huang et al. 2013; Yuwen and Wang 2013; Zeidan et al. 2014). This task has been approached in many
ways by exploring chemical stability of the quantum dots in recent years. One example was thermal and photo stability of CdTe quantum dots tested in the Chasteen research group and reported by Wansapura et al. (2015). Another was a study designed to understand bacterial toxicity of quantum dots and reported by Monrás et al. (2014). The term decomposition may be substituted with the term biodegradation when the decomposition occurs in biological systems with which the QDs are interacting.

**QD Biointeraction**

Since many QD-stability experiments with QDs interacting with biological systems have not been reported in detail so far, this research undertakes a systematic experimental study of QD interaction with bacteria. The biodegradation of quantum dots by bacteria may be described as their lack of stability to resist decomposition, resulting in physicochemical changes.

In this study, the decomposition/biodegradation of CdSe quantum dots was investigated by measuring bacterial growth rates of a metalloid-resistant bacterium from Antarctica and an *E. coli* wild type bacterium and by analyzing bacterial headspace (HS) gases for Se-containing volatiles—organo-selenium gaseous compounds—produced by bacteria during their interaction with the quantum dots in growing liquid cultures. The presence of Se-containing volatiles in the bacterial headspace is potential evidence for the biodegradation of quantum dots by the culture’s bacteria because there is no selenium-containing source other than CdSe quantum dots in those experiments beyond the LB medium’s trace amount of Se, <1 ppm Se (Pathem 2007).

Tharaka Wansapura, a member of the Chasteen research group, in conjunction with our Chilean collaborators, previously described the thermal- and photo-stability studies of
capped CdTe QDs under various storage conditions for several days (Wansapura et al. 2015; Wansapura, 2014). They reported no decrease in photoluminescence in dried, powered QD samples stored for 76 days. In the recent work of Kaur and Tripathi (2014), the stability of the mercaptoacetic acid capped CdSe QDs was investigated over a range of pH (4–11) and they report that QDs can be applied in a 6-9 pH range without affecting the QD stability. Silica-polymer dual layer-encapsulated CdSeZnS QDs have been studied for their stability against a wide range of chemical conditions including strong acids. Hu and Gao reported that the encapsulated QDs constructed using their technique were more stable than those from other techniques, and also suggest that their QDs may have good biocompatibility for in vivo applications (Hu and Gao 2010). Other research focused on photochemical and photophysical properties of CdS QDs that were improved using colloidal carbon spheres coating—their research goal: synthesis of non-degradable QDs for industrial wastes. The coated QDs were successfully employed in the process of dye-degradation in waste water and QDs were stable after the experiments (Hu et al. 2010).

Scenario of Bio-Nano-Interactions

This study primarily focused on bacterial interaction with QDs and the relevant experiments (culture studies) were designed accordingly, but it is useful to consider some important facts about bio-nano-interactions reported in the fields of nanotoxicology and drugs synthesis. One study (Gupta et al. 2011) suggested that a key issue was subcellular nano-bio-interactions because the dimensions of the nano objects and the subcellular level cells in the bacteria, in the human, or in any biological system are in the nanometer range. The interactions at the nano-bio interface should be viewed carefully with the diversity of nano objects which, in general, vary with size, surface charge, surface area, surface coating,
morphology, concentration and composition, and impurities (Cauerhff et al. 2013; Nel et al. 2009). In addition, Gatti and Montanari (2014) distinguished a few types of nano-bio-interactions considering nano object interactions at cellular levels in vitro:

- the interaction with the extra cellular matrix or medium;
- the interaction with the cellular membrane and sensors;
- the interaction with the cytoplasmic components and organelles;
- the interaction with the nucleus.

All those in vitro experiments were suitable to verify the nano-bio-interactions (Gatti and Montanari 2014); however, our study is more fundamental and examines bacterial/QD interaction in only a general way, focusing on toxicological experiments and detection of metalloid-containing breakdown products.

**Nanoparticles (NPs) and QDs**

NPs, also known as nanocrystals, in this field of study are defined by the National Nanotechnology Institute (Glossary NNI Web):

Nanoscale semiconductor crystals “are aggregates of anywhere from a few hundred to tens of thousands of atoms that combine into a crystalline form of matter known as a ‘cluster.’ Typically around ten nanometers in diameter, nanocrystals are larger than molecules but smaller than bulk solids and therefore frequently exhibit physical and chemical properties somewhere in between. Given that a nanocrystal is virtually all surface and no interior, its properties can vary considerably as the crystal grows in size.”
Yuwen and Wang (2013) note that whenever the semiconductor nano particles sizes are smaller than their bulk exciton Bohr radii (1-10 nm) the motions of the holes and the electrons (movements) are restricted within the three dimensional space. And also they describe that there are discrete energy levels created in the nanocrystals upon splitting its continuous energy band. They named such semiconductor materials *quantum dots (QDs).*

**Glutathione-Capped CdSe QDs**

When the QD synthesis involves colloidal chemical routes, whether organic-phase synthesis or aqueous-phase synthesis (Yuwen and Wang 2013), typically there are concerns and issues encountered with the nanocrystals. These are mainly 1) how to control the nucleation and growth (Yuwen and Wang 2013) and 2) how to increase water solubility and biocompatibility (Pérez-Donoso et al. 2012; Huang et al. 2013). To address these issues, a surface coating or a cap has been introduced with materials such as L-glutathione (Monrás et al. 2014) or silica (Zeidan et al. 2014). The constituent of the capped or surface-layer coated QDs is a CdSe core plus a surface layer. The basic inherent photo properties (optoelectronic, tunability) of QDs are determined by the CdSe core and particle size, and their toxicity and solubility properties are affected by the surface layer (Hu et al. 2010; Hu and Gao 2010; Kaur and Tripathi 2014; Monrás et al. 2014). QDs used in this study were composed of CdSe (inner core) with glutathione (GSH) as a capping agent (surface layer).
Core of the QDs: Cadmium and Selenium

Cadmium (Cd)

Cadmium (Cd) is as abundant as 0.16 ppm in the earth’s crust (Greenwood and Earnshaw 1998). It is found mostly in zinc ores and as CdS, in greenockite. Among cadmium compounds, chalcogenides CdS (zinc blende crystal structure) and CdSe (Wurzite crystal structure) are very important because they are more stable in hexagonal forms. The major application of cadmium is in batteries and coatings. Also CdS is used in thermally-stable pigments and stabilizers in PVC (Greenwood and Earnshaw 1998). Cadmium(II) chalcogenide compounds (CdS, CdTe and CdSe)—composed of the elements of Group 12 (Zn, Cd, Hg) and Group 16 (S, Se, Te)—exhibit useful physiochemical properties, such as a wide range of band gaps (0 ~4 eV) and large exciton binding energies that are very useful in solid-state industry applications (He and Zhang 2013).

Selenium (Se)

Selenium is rare, the 66th most abundant element (50 ppb) in the earth’s crust (Greenwood and Earnshaw 1998). Elemental selenium (Se0) and oxyanions of selenium [selenite (SeO3^2-) and selenate (SeO4^3-)] are the most common inorganic forms in the biosphere. Selenium is a required trace-nutrient for many biological systems. For adult humans, the minimum required level is about 50 µg per day and the upper limit level is ~400 µg per day (Patching and Gardiner 1999; El-Bayoumy 2001). Chasteen and Bentley (2007) summarized the general toxicity order of selenium species found in the environment: (SeO3^2-) > (SeO4^3-) > organo-Se > elemental (Se0). When the minimum
required daily dietary level is exceeded by approximately 25 times selenium toxicity begins (Chasteen and Bentley 2007; Patching and Gardiner 1999).

**Biological Forms of Selenium**

Selenium and sulfur are in the same group in the periodic table and therefore there are many similarities in the chemical properties of the two elements and many of their compounds. Some biologically important compounds of selenium are sulfur analogues (Patching and Gardiner 1999; Schwarz and Folz 1958). Selenium can be found in amino acids and in proteins and enzymes in the human body. The selenium analogue of cysteine, selenocysteine (majority of biological selenium), is called the 21st amino acid (Bock et al. 1991); the selenium analogue of methionine, selenomethionine, is incorporated into albumin and hemoglobin in plasma and red blood cells, respectively (Patching and Gardiner 1999). Selenoproteins containing the selenocysteine amino acid in their active sites make the protein more reactive when compared to the sulfur analogues. Glutathione peroxidase and selenoprotein P are two of the other identified selenoproteins. Glutathione peroxidase (an enzyme) is a very important protein which helps to protect the cell components of the human body from the reactive oxygen species hydrogen peroxide (Patching and Gardiner 1999).

**Other Constituent of the QDs: Sulfur (S)**

Carbon and sulfur were the earliest known non-metal elements to the ancients. Sulfur is found as the 16th element in abundance (340 ppm) in crustal rocks in mainly uncombined forms. As sulfur is ubiquitous in nature (biological systems, plants, rocks,
ocean and air), it exists in inorganic and organic compounds mainly as sulfides (H₂S and organosulfur compounds such as sulfur-containing proteins), disulfides (S₂²⁻ such as CH₃SSCH₃), elemental sulfur (S⁰, S₈ and S₆), and as sulfate (SO₄²⁻) (Greenwood and Earnshaw 1998). It is not only the most abundant chalcogen in the earth’s surface but also it is an important bio-element in all living organisms. Microorganisms need sulfur to synthesize sulfur-containing amino acids such as cysteine and methionine and vitamins such as thiamine and biotin (Tortora et al. 2010).

**Glutathione-Capped CdSe QD Synthesis**

Recent work in the Chasteen group has involved synthesizing and examining the physical stability of CdS, CdSe, and CdTe glutathione-capped QDs (Wansapura et al. 2015; Rathnaweera 2015; Hettiarachchi 2015). GSH-capped CdSe QDs were synthesized in our laboratory by two different methods: a conventional heating method (Pérez-Donoso et al. 2012; Huang et al. 2013) that was designed by our collaborators in Chile, and a microwave heating method (Hu et al. 2010; Karan and Mallik 2007) that was developed in our lab for CdS QDs (Hettiarachchi 2015).

**Analysis of Bacterial Growth Rates**

**Growth Rates, Logarithmic Growth, and Specific Growth Rates**

The systematic observations of the growth that occurs in life forms are useful quantitative measurements for many purposes; for example, human growth rate measurements (height and weight) of early childhood development are still taken into consideration as key indicators for the healthy growth of a child. The observation of the
phenomenon of growth—via cell light scatter—is often employed for measuring bacterial growth (as biomass) in bacterial cultures, and these quantitative measurements are transformed into graphical representations as bacterial biomass changes with time—so called bacterial growth curves. To obtain a successful growth rate experiment, there are some minimum requisite conditions to be fulfilled such as a viable inoculum, nutritional medium, requisite amounts of oxygen, temperature control, etc. (Pirt 1975).

Given that the requirements for growth are satisfied, during an infinitely small time change ($dt$), a small increase in biomass ($dx$) is directly proportional to the biomass ($x$) present at time of measurement and the time interval. It is given below as:

$$dx = \mu \cdot x \cdot dt \quad (1)$$

Rearranging

$$\frac{dx}{dt} = \mu \cdot x \quad (2)$$

$\frac{dx}{dt}$ can be expressed as the population growth rate. The parameter $\mu$ (h$^{-1}$) is defined as the *specific growth rate* (growth per unit amount of biomass).

When $\mu$ is constant, integration of Eqn (1) give,

$$\ln x = \ln x_0 + \mu t \quad (3)$$

Here $x_0$ is the biomass at $t=0$ and $t$ is some later time, and it follows that

$$x = x_0 \cdot e^{\mu t} \quad (4)$$

This relationship is used to obtain *a logarithmic growth* (curve), a plot of biomass versus time (Pirt 1975).
Growth curves were used in the work reported here as a means of following the putative toxic effects of QDs on bacterial growth.

Amount of Light Scattered/Optical Density at 600 nm

Measuring light scatter is one of the techniques which are used to determine the biomass in live bacterial cultures. This technique makes the measurements instantaneous and convenient to the tester, especially in cultures undergoing continuous biomass change. There is a relationship between light scattering and culture turbidity which, in turn, is correlated to the biomass in the culture. The turbidity begins to be visible in an inoculated bacterial growth medium as the bacterial mass (cell density) increases. Therefore, the turbidity, often termed optical density (OD), is recorded as a measurement of cell population. A wavelength of 600 nm was the wavelength used to record the OD readings in all the photometric measurements in this research.

Now, when \( t = t_T \), where \( t_T \) is some later time after \( t_0 \) then,

\[
OD_{600}^{t_T} = OD_{600}^{t_0} e^{\mu t_T}
\]

(5)

and also, when \( t = t_D \), then, Eqn (4) as,

\[
OD_{600}^{t_D} = OD_{600}^{t_0} e^{\mu t_D}
\]

(6)

where \( t = t_D \) is the biomass doubling time. Therefore, the relationship between Eqn (5) and (6) can be written as,

\[
OD_{600}^{t_D} = 2 \left( OD_{600}^{t_T} \right) = 2 \left( OD_{600}^{t_0} e^{\mu t_T} \right)
\]

(7)
where, analogous to $x_0$, $OD_{600}^{t_0}$ is the initial optical density of a freshly inoculated culture and $OD_{600}^{t_D}$ is the OD$_{600}$ value at $t_D$ and $OD_{600}^{t_T}$ is the OD$_{600}$ value at $t_T$.

By rearranging Eqn (5) and (6), the specific growth rate, $\mu$, is obtained as,

$$\mu = \frac{(\ln OD_{600}^{t_D} - \ln OD_{600}^{t_T})}{(t_D - t_T)}$$

and with Eqn (7),

$$\mu = 0.693 \ln \frac{OD_{600}^{t_T}}{(t_D - t_T)}$$

**Analysis of Headspace (HS) Gases**

**Metabolism of Metalloids**

Chasteen and Bentley (2003) report in their review that inorganic forms of selenium can be converted into organic forms by biomethylation and also describe that (based on the previous studies in biological systems) those methylated forms are generally less toxic than their precursor inorganic forms. This process is considered to be the biological detoxification of selenium. This process may consist of a series of reduction and/or methylation steps that produce either the much less toxic elemental form or the methylated, volatile forms (Ridley et al. 1977; Yu et al. 1997; Swearingen et al. 2004; Burra et al. 2010).

**Headspace Gases of Interest**

There are many organo-sulfur and -selenium volatile compounds that have been identified in the bacterial headspace. Methanethiol (CH$_3$SH: MeSH), dimethyl sulfide (CH$_3$SCH$_3$: DMS), dimethyl disulfide (CH$_3$SSCH$_3$: DMDS), and dimethyl trisulfide
(CH$_3$SSSSH$_3$: DMTS) are volatile organo-sulfur compounds which have been detected in previous bacterial HS analysis. Additionally, Se-containing volatiles/organo-selenium compounds that have been detected previously include dimethyl selenide (CH$_3$SeCH$_3$: DMSe), dimethyl diselenide (CH$_3$SeSeCH$_3$: DMDSe), and dimethyl selenenyl sulfide (CH$_3$TeSCH$_3$: DMSeS) among others (Swearingen et al. 2004; Burra et al. 2010).

**Solid Phase Microextraction (SPME)**

SPME is one of the sample collection techniques used to extract volatile components in the gas phase. It is widely recognized as a convenient, yet reproducible, sample collection method for gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). In SPME, the analytes are absorbed onto a microfiber coating (Carboxen/polydimethylsiloxane in our experiments) (Camarasu 2000; Furton et al. 2000; Swearingen 2005). In addition, the sample extractions were primarily performed using fibers with large diameters—to increase the amount of analyte absorbed—and using liquid culture volumes from 10 mL up to 500 mL to increase analyte HS gases’ concentrations. This has been achieved in this research by using either 16-mL test tubes or Schott flasks with a novel enclosure cap (Stalder et al. 1995). The larger volume device was especially useful for sample collection for GC/MS experiments because GC/MS is relatively insensitive (detection limits of ng on-column) and therefore requires analyzing HS components at relatively high concentrations (Swearingen 2005).
Separation and Detection of HS Gases Using GC and GC/MS

Gas chromatography (GC) is widely used in the separation of the methylated chalcogens. A fluorine-induced chemiluminescence detector coupled to a gas chromatograph has been used to sensitively determine organo-sulfur and -selenium headspace compounds with detection limits on the order of picograms on-column (Burra 2009; Swearingen et al. 2004). For the HS gases analysis carried out in this work, the HS volatile samples were prepared from the live cultures of the Antarctic strain or a wild type E. coli, amended with different suspensions of GSH-capped CdSe QDs. The amended cultures were grown anaerobically for hours in vials and then pre-concentrated, extracted and injected into the gas chromatograph using SPME fibers. GC/MS was also used for the identification and detection of some HS compounds with cryogenic temperature conditions in order to trap compounds with relatively low boiling points.

Studies and Analysis of Bacterial Interaction with QDs

In order to examine the biological stability of GSH-capped QDs, this research primarily focused on 1) the studies of bacterial growth curves in cultures amended with different amounts of QDs and 2) the analysis of HS gases (after the initial phase of the research), particularly organo-Se-containing volatiles produced by the bacterial effects after the QDs interactions. Different concentrations of suspended QDs were mixed with the inocula growing aerobically on a microwell plate and then absorbance/scattering measurements were recorded. The HS gases produced from the inocula growing anaerobically with different QDs suspensions were extracted and concentrated using solid phase microextraction. This was followed by GC separation and GC/MS analysis for the
organo-sulfur and -selenium HS compounds (Burra 2009; Burra et al. 2010; Swearingen et al. 2004).
CHAPTER II
MATERIALS AND METHODS

REAGENTS

The chemicals used in this research include: cadmium chloride hydrate (CdCl$_2$·xH$_2$O; Sigma-Aldrich, Saint Louis, MO, USA), sodium selenite (Na$_2$SeO$_3$; Sigma-Aldrich), sodium borate (Na$_2$[B$_4$O$_5$(OH)$_4$]·8H$_2$O; Sigma-Aldrich), sodium citrate (Na$_3$C$_6$H$_5$O$_7$·2H$_2$O; Fisher Scientific, Fair Lawn, NJ, USA), L-glutathione reduced (GSH) (Sigma-Aldrich or Alfa Aesar, Ward Hill, MA, USA), Luria-Bertani (LB) medium (tryptone, yeast extract, and sodium chloride), ethyl alcohol (C$_2$H$_5$OH; Pharmco, Brookfield, CT, USA), and dimethyl disulfide (CH$_3$SSCH$_3$; Sigma-Aldrich). All chemicals were used with no further purification.

EQUIPMENT AND INSTRUMENTATION

Laboratory equipment for preparation, separation, and processing of QDs and bacterial cultures for this study included: Eppendorf and Falcon® tubes (15 mL and 50 mL; VWR, Radnor, PA, USA), shaker flasks (Polycarbonate-250 mL; Triforest, Irvine, CA, USA), and Schott flasks (100–1000 mL; VWR) for bacterial culture storage and growth; a desiccator, a water bath, and an incubator with shaking in variable speed for aerobic culture incubation (VWR); a microwave oven (850 W; Galanz, Naperville, IL, USA) and a microwave digestion vessel (Model 4781, 23-mL volume; Parr Instrument Company, Moline, IL, USA) for QD synthesis; a vortex mixer (Genie 2, Fisher Scientific) for mixing reagents quickly and vigorously; a centrifuge (5810 R; Eppendorf AG, Hamburg,
Germany) for QD separation; an autoclave (Tattnauer; Brinkmann Instruments, Westbury, NY, USA) or a 0.2-μm syringe filter (Pall Corporation, Ann Arbor, MI, USA) for sterilization of growth media; and a digital camera (Coolpex S 9300; Nikon USA, NY, USA) used for capturing images of the QDs and the cultures. All DI water used came from a water purification system, RiOs 3 (Millipore; Billerica, MA, USA) located in the Department of Chemistry at Sam Houston State University.

Instrumentation for the study included: a UV transilluminator (2UVTM UVP; Upland, CA, USA) used for fluorescence screening of QDs and QD-amended cultures; a fluorescence spectrophotometer (F-4500; Hitachi, Schaumburg, IL, USA) used for measuring fluorescence intensities of QD stock suspensions; a UV/vis spectrophotometer (V-550; Jasco, Easton, MD, USA) and a Varioskan™ Flash (Multimode reader; Thermo Fisher Scientific, Vantaa, Finland) used for spectral scanning and optical density readings; and capillary GC with a desktop computer (Model 5890 Series II; Agilent Technologies, Wilmington, DE, USA) with a sulfur chemiluminescence detector (SCD) (Model 300; Ionics Instruments, Boulder, CO, USA) (Van Fleet-Stalder and Chasteen, 1998) or a gas chromatography/mass spectrometer (GC/MS) coupled with a (70-eV electron impact) mass selective detector (Agilent model 5973) used for bacterial headspace gases separation, detection, and identification. The SCD’s photomultiplier tube signal was collected and scaled by a picoameter (Model 616; Keithley Instruments, Cleveland, OH, USA). The picoammeter’s 1 V output was wired into the analog to digital board of the desktop computer running Chemstation software.
METHOD PART I: CdSe QD Synthesis and Bacterial Culture Preparations &

Growth Conditions

Synthesis of CdSe QDs

GSH-capped CdSe QDs were synthesized in our laboratory by two different methods: a conventional heating method (Pérez-Donoso et al. 2012; Huang et al. 2013) that was designed by our collaborators in Chile, and a microwave heating method (Hu et al. 2010; Karan and Mallik 2007) that was developed in our lab for CdS QDs (Hettiarachchi 2015). The stock solutions of buffer, metals, selenite and capping agents were prepared initially as follows:

Borax Citrate Buffer Solution

The stock solution of borax citrate solution (37.50 mM) was prepared by mixing sodium borate (3.576 g) and sodium citrate (2.758 g) and dissolving in a 250-mL volumetric flask filled with deionized water up to the mark (pH=9). The pH was verified with a calibrated pH meter.

Cadmium Chloride Solution

The stock solution of cadmium chloride (16.7 mM) was prepared by dissolving cadmium chloride hydrate (0.916 g) in DI water in a 250-mL volumetric flask.
**Glutathione (GSH) Solution**

The solution of GSH (50 mM) was prepared by dissolving GSH (0.792 g) in DI water in a 50-mL volumetric flask.

**Sodium Selenite Solution**

The stock solution of sodium selenite (5 mM) was prepared by dissolving sodium selenite (0.219 g) in DI water in a 250-mL volumetric flask.

**Hydrothermal Method of QD Synthesis**

At first, an exact volume of cadmium chloride (2 mL) and borax citrate solution (4 mL) from the stock solutions and GSH (2 mL) from that freshly-prepared solution were mixed in the test tubes (the number of test tubes varied) and then the test tubes were vortexed vigorously for one minute. Next sodium selenite (2 mL) from the stock solution was added to each test tube. At last, the test tubes, capped, were incubated at 90 °C for two hours and after the incubation period, allowed to cool to room temperature. The fluorescence emissions of QD suspensions in the test tubes were detected by the UV transilluminator with excitation at 365 nm.
**Microwave Irradiation Method of QD Synthesis**

As described for the hydrothermal method in the previous paragraph, the same volumes of the chemicals were mixed for a different set of test tubes. The same procedure was followed up until the incubation step, and then the whole contents of a test tube (one at a time) were poured into the microwave digestion vessel, the container capped and microwaved for 15 s at 850 watts. After cooling, the room temperature samples were examined for fluorescence with the UV transilluminator at 365 nm.

**Harvesting Powdered CdSe QDs**

The aqueous synthetic mixture of CdSe QDs (10 mL) was transferred into a Falcon tube and an aliquot of ethanol (20 mL) was added to the tube and then the mixture was centrifuged at 12000 rpm for 20 minutes at room temperature. After decanting the liquid carefully, CdSe QDs were separated and dried for 24-48 hours in the desiccator over fresh anhydrous calcium sulfate at room temperature. The product was powdered QDs.

**Washing CdSe QDs**

A mixture (3 mL) from a solution containing borate-citrate buffer solution (5 mL), and ethanol (10 mL) was used to wash the QDs in order to remove unreacted loosely-bound selenite ions in an effort to minimize any free Se on QD surfaces. After washing once, the QDs were dried in the desiccator for 24 hours.
**CdSe QDs for the Experiments and Total Luminescence Spectra**

Four different concentrations of CdSe QDs suspensions (1000/1500/2500/3750 ppm) were prepared by suspending CdSe QDs (10/15/25/37.5 mg), respectively, in a 10-mL volumetric flask filled to the mark with freshly prepared (sterilized) LB medium. Total luminescence spectra were taken from the samples of the suspended concentrations (1000/1500/2500/3750 ppm) using with fluorescence spectrometer scanning 200 nm to 900 nm range for excitation and for emission. Note that all QD amended concentrations discussed in this thesis actually refer to QDs suspended in solutions, not actually dissolved.

**Bacterial Culture Preparations & Growth Conditions**

The bacterial cultures of A0LBCd strain and *E. coli* BW 25113 (from live cultures grown from agar plates) were used in all experiments; they were prepared in a complex growth medium (LB) for the growth curve experiments and the headspace (HS) gas analysis experiments by incubating aerobically/anaerobically (Pirt 1975; Tortora et al. 2010). A0LBCd, a cadmium-resistant bacterium, and B6LBTe, a tellurium-resistant bacterium, were isolated from Antarctica and thought to be good organisms to use to explore possible bacterial metabolism of QDs synthesized in this study. BW 24113 was a wild type *E. coli*.

**Preculture Preparations & Growth Conditions**

The live cultures of A0LBCd, B6LBTe strains and *E. coli* (from the agar plates) were mixed with the sterilized LB medium (50 mL) and grown overnight aerobically at 37 °C in shaker flasks (with open-lid caps for air circulation) in the incubator with proper
shaking. These pre-culture solutions were used in subsequent culture (inocula) preparations.

**Culture Preparations for the Experiments & Growth Conditions**

To prepare cultures, first, the pre-cultures of the bacterial strains (inocula) were diluted (1:100 dilutions) with sterilized LB medium (total volume varied).

**For the growth curve experiments**, the diluted cultures in different volumes were loaded into the labeled wells (each well with same total volume) in a sterilized 96-microwell plate (this is described in detail in Part II of the methods). After that, the microwell plate—loaded with the bacterial cells in the LB medium—was inserted into the Varioskan instrument and the bacterial cells were allowed to grow at 37 °C aerobically at 240 rpm (revolution per min) for a programed period of time until the desired optical densities were achieved in the culture solutions.

**For HS gas analysis experiments**, the diluted bacterial preculture volumes (usually 100-250 mL) in shaker flasks (with the closed lid caps) were grown anaerobically at 37 °C in the incubator with shaking and then allowed to grow until the desired optical density range (0.5 to 0.7) before the inocula were transferred to test tubes (16 mL) or vials with specific QD suspension concentrations.
METHOD PART II: Time Course Experiments

Varioskan Experiments

The Varioskan instrument was used to obtain optical density (OD) measurements—photometric readings (absorbance) at 600 nm—of the bacterial cell populations in the cultures and the inocula amended with the different CdSe QD concentrations grown in the wells in the microplate. The experiments were designed for 12/14/19/48 h time courses for different CdSe QDs concentrations (333 ppm/1000 ppm/1500 ppm) and the instrument was programmed accordingly to record the absorbance at 600 nm (OD$_{600}$) of the changing cells populations in the wells automatically throughout the given time period. In each program, there were two different plate-out time periods (2-4 minutes in each time) for the addition of QD volumes to the cultures which had reached 0.5 OD$_{600}$ (Monrás et al. 2014) and the sterilized LB volumes to the corresponding control cultures (to maintain equal total volumes in each well) and to the control cultures (QD-free). Figure 1 shows how the specific wells (12 wells in triplicate) were loaded with all volumes including three replicates of blank (BL medium), QDs (1000/1500 ppm in the wells), control cultures (A0LBCd and BW), and the same cultures amended with QDs. The rest of the wells (12x5) were loaded with deionized water. The total volume (300 µL) was maintained in each well while adding the CdSe QDs (120/180 µL of 2500 ppm) and LB medium (180/120 µL) volumes, respectively, to the designated wells.
Fig. 1 A microplate layout used for one of the experiments. Well content and their filled volumes are detailed.

**Growth Rate Curves**

The Varioskan recorded all OD<sub>600</sub> changes in the selected wells throughout the given time period for the experiment. The growth curves were obtained by tabulating the natural-log values of OD<sub>600</sub> [ln(OD<sub>600</sub>)] against time (minute/hours) and the specific growth rates of the bacteria of interest were calculated from ln(OD600) values and cell-density doubling data.
METHOD PART III: Analysis of Headspace Gases Using GC and GC/MS

Headspace Gas Analysis

Headspace Sampling

In all headspace samples, the liquid content was maintained with a total volume (10 mL) in each separate 16 mL test tube sample. There were 10 mL liquid samples of the blank, GSH-capped CdSe QDs in LB, the control cultures or the inocula (amended with specific QD concentrations) in the test tubes capped with open-top screw caps with Teflon/silicon-lined septa (Alltech, Deerfield, IL, USA). Therefore, the headspace above the liquid content was the same volume in each test tube. At the beginning, the volume (10 mL) of the LB and CdSe QDs was transferred into two separate test tubes, and then the control cultures (10 mL) of A0LBCd or BW 25113 at 0.5 OD$_{600}$ were transferred into another two (at least one for each culture) separate test tubes. In the next step, the same cultures mixed with QDs were transferred into other separate test tubes (at least one for each culture) after adding specific GSH-capped CdSe QD volumes into the test tubes, maintaining the total volume (10 mL) in each case. All test tubes were closed with the cap and septa immediately after transferring the whole contents. Finally, all the test tubes were placed in the water bath at 37 °C and incubated for 48 hours before the HS gas analysis because the volatile HS production for all experiments were examined with cultures in stationary phase (over more than 24 hours). This experiment set-up restricted the bacteria to grow anaerobically after the initial aerobic preculture stage.
**SPME Sampling**

Bacterial HS volatiles were concentrated, extracted and collected by 75-µm carboxen-polydimethylsiloxane (PDMS) SPME fibers (Supelco, Bellfonte, PA, USA). The absorption time was 15 minutes and the temperature was usually 37 °C; however, in the case of the larger HS volumes, some experiments used 45 °C in an effort to increase HS concentrations. The samplings were carried out by the exposure of the fiber in the HS and allowing it to extract the volatiles for 15 min by piercing the septum of the 16-mL test tubes with the SPME needle and retracting the needle to expose the fiber in the sample’s HS. The fiber-extracted HS samples were transferred to the gas chromatograph through the hot GC injector’s septum and the fiber in the SPME was kept on the injector for the entire GC run (20 min). A new fiber was conditioned and thermally cleaned for use according to the data sheet (T794123S, 1999 Sigma-Aldrich Co.) provided by Supelco. And also, the fiber was exposed to air and checked for carryover by making fiber check runs between each sample run.

**GC with Fluorine-Induced Chemiluminescence Analysis**

An SCD was used for the HS gas analyses. A capillary column (15 m length, 320 µm internal diameter) with nonpolar DB-1 stationary phase (100% dimethylpolysiloxane, 5.0 µm film thickness) was used (J&W Scientific, Folsom, CA, USA). The carrier gas was ultrahigh purity helium (UHP) with a carrier flow rate of 1 mL per min and the injection mode was splitless for all experiments. The temperature program used for GC/SCD experiments was 30 °C at the inception with 2-min holding time and then
ramped to 120 °C at the end at 5 °C per min. The gas chromatograph injector temperature was 280 °C and the injector’s split valve opened after one minute to improve peak shape.

**GC/MS Analysis**

Additional HS gas analysis was achieved by a HP 5890 gas chromatograph coupled with a HP 5973 mass selective detector with a positive electron impact ionization source (70 eV). UHP He (1 mL/min) was used as carrier gas in this gas chromatograph with an Agilent DB-5 column (5% phenyl 95% methyl polysiloxane, 0.32 µm internal diameter, 1.4 µm film thickness, 30 m length). No solvent delay was used and the mass spectrometer’s mass scan range was selected to eliminate unwanted low mass fragments, for instance, a range of 29–300 m/z. Two temperature programs were used for the GC/MS experiments. One program was used for cryogenic conditions (-20 °C) which was accomplished with liquid nitrogen plumbed into the gas chromatograph’s oven. Therefore, the temperature, at the beginning was -20 °C with 5 min hold time and ramped to 100 °C at 5 °C per min at the end; and in the second temperature program used, the temperature started at 30 °C with 2 min holding time, and then ramped to 100 °C at 5 °C per min, and then ramped to 200 °C at 15 °C per min at the end. All injections were splitless mode in the 275 °C injector. The large volume Schott flasks with a novel enclosure cap (Stalder et al. 1995) were especially useful for sample collection for GC/MS. The SPME fiber and headspace sampling procedure were the same as used for GC/SCD.
CHAPTER III
RESULTS

Fluorescence Studies of the GSH-Capped CdSe QDs

The Colors of the Freshly Prepared CdSe QDs in the Synthetic Mixtures

There was no difference in color (slight green-yellow) for the QDs (capped CdSe) prepared in the thermal and microwave irradiation methods, but they fluoresced different colors (greenish yellow to bright yellow) under UV light at 365 nm as shown in Fig. 2.

Fig. 2 Color variation—green to yellow—of fluorescence observed under UV 365 nm irradiation for powered QDs (A) and QDs synthetic mixtures prepared with the thermal (B) and microwave irradiation (C) methods.

Fluorescence of the GSH-Capped CdSe QDs Prepared for the Experiments

Photographs of fluorescence under UV 365 nm are shown in Fig. 3 below for the experimental QD suspensions of different concentrations which were prepared as stock suspensions and for intensity comparison purposes.
Different colors of fluorescence were observed (under UV 365 nm) from the suspended QDs concentrations (3750/2500/1500/1000/500/333 ppm), prepared in sterilized LB medium.

**Fluorescence Spectroscopic Details of GSH-Capped CdSe QDs**

Total luminescence spectra (Fig. 4) were taken for the freshly-prepared stock suspension of QDs (3750/2500 ppm) and for the freshly-prepared QDs suspended concentrations (1500/1000 ppm) in the LB sterilized medium.
Fig. 4 The total luminescence spectra of four different suspended QD concentrations (3750/2500/1500/1000 ppm).

As shown in the figure above (Fig. 4), there are prominent fluorescence peaks and a line of scattering peaks, which stretches diagonally throughout the scanned range for each concentration. The most predominant peak—the highest intensity—in each concentration shows the excitation wavelength (350-500 nm) and the emission wavelength (500-575 nm).
and the second highest peak appears in the near infrared region, with approximately 800 nm excitation wavelength and 450 nm emission wavelength.

**Bacterial Growth Curves Experimental Results**

For the construction of the growth curves and their analysis, the absorbance of each sample at 600 nm (OD$_{600}$) was recorded using the Varioskan and those data were used to construct the bacterial growth curves. The resultant growth curves obtained for the control cultures of A0LBCd, BW wild type (*E. coli*) and B6LBTc bacteria strains are illustrated in Fig. 5, and GSH-capped CdSe QDs (333 ppm) and (1000/1500 ppm) amended cultures of A0LBCd and BW are shown in Fig. 6 and Fig. 7, respectively.

It is important to note that for Varioskan experiments, two or more replicate samples were used to obtain better precision of the readings; therefore, the standard deviation was calculated and displayed with vertical-error bars which may not be clearly visible in some places due to blockage from the markers employed in the plotted curves.

To examine the possible effect of oxygen limitation on bacterial growth in the Varioskan experiments, growth curves were generated for the three bacterial strains under conditions with or without the microplate covers (Fig. 5).
Fig. 5 The growth curves were obtained from different bacteria grown in a 96-well plate with cover (top) and without cover.
Fig. 6 Bacterial growth curve of control cultures and QD-amended cultures (333 ppm) for a 12 h Varioskan experimental time period.
Fig. 7 Bacterial growth curves of the control cultures and QD-amended cultures 1000 ppm (top) and 1500 ppm for a 48 h Varioskan experimental time period.
Calculations of Specific Growth Rates

The specific growth rates of the bacteria used for the above were calculated according to Pirt (1975) and the results are displayed in Table 1.

Table 1.
Specific growth rate results.

<table>
<thead>
<tr>
<th>Bacteria Strains</th>
<th>Specific Growth Rates With a Cover</th>
<th>Specific Growth Rates Without a Cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>A0LBCd</td>
<td>0.222±0.013/h</td>
<td>0.263±0.007(0)/h</td>
</tr>
<tr>
<td>BW</td>
<td>0.028±0.001/h</td>
<td>0.040±0.001(6)/h</td>
</tr>
<tr>
<td>B6LBTc</td>
<td>0.023±0.001/h</td>
<td>0.031±0.000(4)/h</td>
</tr>
</tbody>
</table>

It is important to note that all QDs amendments were carried out when the relevant bacterial culture reached a desired optical density of 0.5 at 600 nm in the designated well of the microplate. In the case of the growth curves of 1000 ppm and 1500 ppm, the points at which the QDs were incorporated (after 150 min) to the A0LBCd cultures may not be clearly visible in plots displaying those data due to the crowding of data points in each graph at the beginning. In addition, at the very inception of the same graphs, overlapped data points can be seen.

Headspace Gas Analysis Results

In this study, the bacterial HS gases analyses were usually carried out after 48 h using GC-SCD and GC/MS. The color changes of HS samples (liquid portions of the test tubes) of A0LBCd and E. coli control cultures and cultures amended with CdSe QDs were visualized under different light conditions in a 48 hour-incubation period. Some of the observed changes in color and precipitation are displayed below in Fig. 8. All test tubes
samples [Fig. 8(A) through 8(E)] contain A0LBCd bacterial culture solutions amended with GSH-capped CdSe QDs.

![Image](image.png)

**Fig. 8** BW bacterial cultures with QD amendments illuminated by either daylight or UV (365 nm). A: daylight; left, 0 time; right, after 10 h incubation. B: UV; left, 0 time; right, after 10 h. C: daylight, after 24 h. D: UV, after 24 h. E: UV, after 48 hours.

The left test tubes in Fig. 8(A), observed under daylight, and Fig. 8(B), under UV light, displayed yellow shades at the beginning of the experiment, immediately after the QDs addition. The right test tubes in 8(A) and 8(B) displayed differences in shades (in their liquids) under the same light sources for the same bacterial cultures after ten hours of incubation at 37 °C. More and more precipitation was observed after 24 and 48 hours [Fig. 8(C-E)].

Fig. 9 displays the SCD chromatograms of a newly-cleaned fiber exposed only to lab air runs (a fiber check), HS analyses of LB media incubated anaerobically at 37 °C after sterilization either via autoclaving or sterile filtering; and unwashed QDs in autoclaved LB incubated for 48 h.
Fig. 9 Chromatogram of fiber check (A), HS samples of autoclaved sterilized LB (B), sterile-filtered LB (C), and unwashed QDs (200 ppm) incubated in autoclaved LB after 48 h (D).

Experiments were carried out to examine the headspace of bacterial cultures incubated after amendment with QDs or without. Experiments also examined the differences in HS composition for cultures amended with QDs that had been washed with a buffer/ethanol solution versus those that had not.

Fig. 10 displays the SCD chromatograms obtained from HS samples of A0LBCd and BW cultures grown anaerobically without CdSe QDs for a 48 h incubation period.
Fig. 10 SCD Chromatograms of A0LBCd (top) and BW bacterial control cultures grown anaerobically in LB medium without CdSe QDs for 48 h.

Fig. 11 and 12 show the SCD chromatograms of HS components of A0LBCd and BW cultures amended with CdSe QDs (1000/1500 ppm) and incubated for 48 h.
Fig. 11 Chromatograms of bacterial headspace for BW cultures amended with unwashed CdSe QDs: 1000 ppm (top) and 1500 ppm.
**Fig. 12** Chromatogram of bacterial headspace of the unwashed CdSe QDs (1500 ppm) amended to A0LBCd culture in LB medium.

**Fig. 13** Chromatograms of headspace samples from washed CdSe QDs (1000 ppm) added to A0LBCd (top) and BW cultures in LB medium.

Fig. 13 contains SCD chromatograms generated from HS samples of A0LBCd and BW cultures amended with washed QDs after 48 h.
Fig. 14 illustrates the TIC and mass spectrum of methanethiol (MeSH) obtained from HS produced by BW culture amended with CdSe QDs (1000 ppm) for 48 h.

Fig. 14 GC/MS TIC (top) and mass spectrum from a chromatographic peak eluting at 1.43 min (methanethiol, MeSH) from a headspace sample of a culture of BW amended with CdSe QDs (1000 ppm) and incubated for 48 h.
Fig. 15 and 16 illustrate the TIC and mass spectrum of dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) obtained from HS of A0LBCd cultures amended with QDs (1000 ppm).

**Fig. 15** GC/MS TIC (top) and mass spectrum from a chromatographic peak eluting at 3 min (dimethyl disulfide, DMDS) from a headspace sample of a culture of BW amended with CdSe QDs (1000 ppm) and incubated for 48 h.
Fig. 16 GC/MS TIC (top) and mass spectrum from a chromatographic peak eluting at 5.0 min (dimethyl trisulfide, DMTS) from a headspace sample of a culture of BW amended with CdSe QDs (1000 ppm) and incubated for 48 h
Fig. 17 and 18 illustrate the TIC and MS spectral details of some organic compounds in the HS produced by BW cultures growing with QD amendments (1000 ppm) for 72 h.

**Fig. 17** GC/MS TIC (top) and mass spectrum from a chromatographic peak eluting at 4.8 min (ethyl acetate) from a headspace sample of a culture of BW amended with CdSe QDs (1000 ppm) and incubated for 72 h.
Fig. 18 GC/MS TIC (top) and mass spectrum from a chromatographic peak eluting at 6.7 min (heptane) from a headspace sample of a culture of BW amended with CdSe QDs (1000 ppm) and incubated for 48 h.
CHAPTER IV
DISCUSSION

My main interest in this research project was to investigate the interaction of CdSe QDs with certain bacterial strains and to study whether those bacteria could decompose QDs in LB media in growing cultures. For that purpose, GSH-capped CdSe QDs were synthesized and cultures of bacteria strains—A0LBCd, BW (E. coli wild type) and B6LBTe—were grown in LB medium. QD-amended cultures were examined for visual degradation of QDs and the cultures’ headspace was analyzed for organo-selenides that had been detected in previous work with biomethylating bacteria.

GSH-Capped CdSe QDs

The QDs were synthesized in our laboratory by the conventional heating method (Pérez-Donoso et al. 2012; Huang et al. 2013), the technique designed by our collaborators in Chile and also by the microwave heating method (Hu et al. 2010; Karan and Malik 2007), a technique developed in our lab for CdS QDs (Hettiarachchi 2015). As shown in Fig. 2 on page 28, the dried QDs samples (crystals and powders) prepared by both techniques are generally a greenish-yellow color; however, in LB media they fluoresced differently depending upon the method of preparation: yellow fluorescence for QDs synthesized from the microwave method and yellowish green for QDs synthesized from the thermal method. In the case of the microwave synthetic technique, the color of the QDs samples varied with microwave heating time (Hettiarachchi 2015). The QD synthetic
process used for QDs prepared for the degradation studies was ultimately using a 15 s microwave heating time at 850 W.

**Fluorescence Studies of the GSH-Capped CdSe QDs**

CdSe QDs resuspended in sterile LB fluoresced under 365 nm UV, and the intensity and wavelength of emission varied slightly depending upon the amendments (Fig. 3 on page 29). The fluorescence spectra taken for GSH-capped CdSe QDs (3750/2500/1500/1000 ppm QD amendments) re-suspended in LB solutions are shown in Fig. 4 on page 30. The main focus of the fluorometric studies was to examine the relationship between QDs amendments and corresponding fluorescence intensities at different excitation wavelengths and compare those to the colors of QDs under room light. The spectra illustrated that fluorescence intensities were proportional to QD amendments and in general, the fluorescence emission wavelength (500-575 nm) corresponds to yellowish green color QDs under room light and in daylight.

In each total luminescence spectrum of Fig. 4, there are three prominent spectral features: two prominent fluorescence peaks and a line of resonance Rayleigh scattering (RRS) (Luo et al. 2002). The RRS stretches diagonally across the total luminescence spectrum and its excitation and emission wavelengths are identical since this is merely a record of light scattered by the QDs to the detector from the excitation source. The prominent fluorescence peaks (taller peak) in each spectrum shows excitation at 300-450 nm and emission at 550-600 nm; this is QD fluorescence similar to what workers in our research group and our Chilean collaborators have seen before (Hettiarachchi 2015; Rathnaweera 2015; Pérez-Donoso et al. 2012). The other prominent fluorescence peak,
with excitation in the near IR around 850 nm, exhibits emission at 570 nm—an emission wavelength that is approximately two-third of the excitation wavelength. That phenomenon is due to resonance non-linear scattering reported by Luo and coworkers (2002). Neat LB growth medium shows no detectable fluorescence in a total luminescence spectrum using these scan ranges (data not shown), and therefore the features highlighted in Fig. 3 are solely caused by the presence of QDs in these experiments. Extending the explanation, it is reasonable to assume that resonance non-linear scattering could be expected to occur in addition to RRS in the solutions studied, when QDs are re-suspended in LB media, which supplies macromolecules to the medium and supports the formation of macromolecular complexes with CdSe QDs (bio-conjugates), a condition that Luo and coworkers suggest promotes non-linear scattering (Luo et al. 2002).

**Bacterial Growth Curve Results**

According to the bacterial growth curves from control culture experiments (without QDs) using the Varioskan robotic instrument (Fig. 5 on page 32), A0LBCd, one of the two Antarctic strains examined in this study exhibited the highest specific growth rate under the conditions studied, followed by BW and then closely by the second Antarctic strain, B6LBTe (Table 1). A growth rate decrease was observed in these experiments (most notable for BW cultures) when the microwell plate cover was in place apparently creating an oxygen-restricted environment in which these bacteria experienced microaerobic conditions that slowed growth.

Another set of growth curve experiments was performed with A0LBCd and BW cultures grown aerobically (without plate covers) with CdSe QD amendments. The
resultant curves for different QD concentration amendments are shown in Fig. 6 on page 33 (333 ppm) and Fig. 7 on page 34 (1000 and 1500 ppm amendments). The CdSe QD amendments were carried out when each culture reached 0.5 OD_{600} in an effort to approximate the same cell population. While the CdSe QD volumes were added to designated wells, equal volumes of LB liquid were added to wells where there were relevant control cultures in order to compensate for the dilution effect of the additions. Within a few hours after the addition (QD amendments and LB liquid volumes), as displayed in Fig. 6 and 7, bacterial growth rates were similar to the rates before the addition, as reflected by the same relative slope; however, the drastic drops in ODs were observed at the time of each addition because of the dilution effect of the volumes added. If the QD amendments had a significant impact on the bacterial cell growth, a change in growth rate should have been apparent after the additions. On the contrary, growth rates were uniformly reestablished after each QD additions. Therefore, as per Varioskan experiments with 333/1000/1500 ppm CdSe QD concentrations, bacterial growth was not affected by these QD additions. A reasonable explanation for these results—no noticeable effect upon bacterial growth rate upon exposure to QDs over the wide range of QD concentration studied—is that the toxicity of the CdSe QDs prepared for these experiment is very low due to the effective isolation of the toxic elements in these nanoparticles.

**Visual Bacterial Degradation of QDs**

To harvest the volatile bacterial components from HS samples, the bacteria of interest were grown anaerobically for a 48 h incubation period at 37 °C. A typical set of HS experiments was comprised of samples of sterilized LB medium, CdSe QDs added to sterilized LB, the control cultures and QDs amended cultures of A0LBCd, and BW in
16-mL test tubes, all capped with open-top screw caps with Teflon/silicon-lined septa. The B6LBTe bacterial strain was not included in these experiments because A0LBCd, the Antarctic bacterial strain with resistance to Cd, and *E. coli* were selected from the preliminary growth curve experiments (Fig. 5) with the highest specific growth rates (Table 1).

Two HS sample test tubes—a part of the HS experiment performed anaerobically with BW bacterial cultures amended with CdSe QDs (1000 ppm)—were examined under UV light and daylight. Photographs of these in daylight and on the transilluminator under UV are displayed in Fig. 8 on page 36. The left test tube in Fig. 8(A) shows, under daylight, the condition of the liquid portion at the beginning of the HS experiment just after QD addition and the right tube shows the same liquid after ten hours incubation at 37 °C. Fig. 8(B) shows the same test tubes under UV light and reveals that the right test tube after ten hours has started precipitation and apparent degradation. QDs incubated without bacteria under identical conditions show no visual signs of this degradation (not shown). The test tubes in Fig. 8(C-E) show further degradation in the liquid portion after 24-48 h. Most clear, in Fig. 8(E), CdSe QDs were degraded/precipitated by bacteria after 48 h of incubation and the resulting QD accumulation in the bottom of the test tube could be observed by UV light. This degradation mirrors thermal degradation seen in QD samples heated for long periods of time in other work performed in our research group (Hettiarachchi 2015).
**Bacterial Headspace Gas Analysis**

HS analyses of bacterial samples amended with QDs were carried out via capillary gas chromatograph coupled with chemiluminescence detection and GC/MS in an effort to detect the possible volatile organo-selenium compounds that have been produced by metalloid-resistant bacterial amended with soluble metalloidal salts like sodium selenate or selenite (Burra et al. 2010; Chasteen and Bentley 2003; Stalder et al. 1995).

SPME fiber checks, an example is shown in Fig. 9 (A) on page 37, did not show any detectable carryover between chromatographic runs. The detection of DMDS in HS samples of sterilized and sterile-filtered LB, Figs. 9(B-C), is an indication that either this organosulfur compound was formed in both autoclaved LB and sterile-filtered LB media, or is generated in the commercial production of the powered LB reagent, the form of LB that was purchased in bulk and used in this work. A larger DMDS peak in Fig. 9(B) as compared to Fig. 9(C) indicated that even more of this volatile compound was produced in the autoclave heating than was present in sterile-filtered media. DMDS was detected in autoclaved LB solution with QD amendments after 48 h incubation, Fig. 9(D); however, the relative size of the DMDS peak in the sterile QD-amended sample is comparable to that of the autoclaved QD-free LB sample. Therefore, it can be assumed that QDs incubated at 37 °C in sterile LB do not produce significantly more organo-sulfides than are already present in autoclaved LB.

SCD-based HS analyses of A0LBCd and BW bacterial cultures with no QD amendments showed detectable amounts of MeSH, DMDS, and DMTS (Fig. 10 on page 38). Those key organosulfur compounds are produced by bacterial biomethylation
processes (Chasteen and Bentley 2003 & 2007) because LB medium contains the sulfur-containing sources which are available for the biomethylation process in growing cultures.

SCD chromatographic results of bacterial HS components analyses obtained from A0LBCd and BW cultures amended with unwashed GSH-capped CdSe QDs (1000/1500 ppm) grown anaerobically at 37 °C for a 48 h incubation period are displayed in Fig. 11 and 12 on pages 39 and 40, respectively. The physical changes of these HS samples (two test tube samples of the BW experimental set) for the 48 h time period were illustrated in Fig. 9(A-E), and discussed earlier. Many of the volatile compounds generated by BW cultures amended with QDs (1000/1500 ppm) and A0LBCd culture amended with QDs (1500 ppm) and detected in HS via SCD are known bacterially-produced organo-sulfur and -selenium chemical species. Selenium-containing compounds detected in those runs (identification based on commercial standards or previous GC/MS experiments, not shown) include dimethyl selenenyl sulfide (DMSeS) and dimethyl diselenide (DMDSe). Other organoselenium compounds such as dimethyl selenide (DMSe), dimethyl diselenenyl sulfide, and dimethyl triselenide (DMTSe) have been reported previously in microbial cultures amended with selenium oxyanions (Stalder et al. 1995; Burra 2009; Burra et al. 2010). Unlike sulfur, which is present in LB, selenium was not added or available (<1 ppm) in the LB medium (Pathem 2007). As Figs. 11 and 12 indicate, at least one selenium-containing HS compound was detected when unwashed QDs were added to growing bacterial cultures, but were not present in sterile LB-only cultures when unwashed QDs were added (Fig. 9). Therefore it can be concluded that the bacteria have either bioprocessed the loosely bound selenide or selenite ions (left over from the synthesis) bound to QDs surface to generate selenium-containing HS species or have degraded the
QDs, releasing selenium from those nanoparticles for bioprocessing. Therefore we designed experiments to test this hypothesis. Growing A0LBCd and BW cultures were amended with washed GSH-capped CdSe QDs and incubated for 48 h anaerobically. Then the HS was sampled and examined for the presence of organo-selenium species (Fig. 13 on page 40). No organo-selenium components were detected in those SCD chromatograms of HS above those cultures incubated for 48 h. Either no volatile organo-selenium species were generated or their concentrations were lower than the detection limits for this instrument for this family of Se-containing compounds (~50 pg on-column). Therefore, according to these results, we can conclude that washed GSH-capped CdSe QDs were stable enough in these experiments and they were not subject to bacterial degradation in a manner that released Se that could be bioprocessed by bacteria under these conditions.

**TIC and Mass Spectra**

Since there were so many different HS compounds in cultures amended with unwashed QDs, additional analyses of HS of controls and QD-amended bacterial samples were carried out using GC/MS. This method allows for the identification of HS compounds that were otherwise invisible to the selective SCD detector via mass spectral data; however, the GC/MS method required much higher analyte concentrations due to its higher detection limits compared to SCD. GC/MS was not able to detect any selenium species in the HS samples. For this family of compounds its detection limits is in the nanogram range and the mass collected via SPME HS sampling and delivered into the GC injector of organometalloid-containing compounds—as detected via SCD—were in the picogram range. A comparison of DMDS detection limits (3σ/N) using a commercial standard for
the two methods showed that the SCD was 80X more sensitive than the GC/MS for this compound (data not shown). Efforts to increase the concentrations of volatile HS compounds using larger liquid culture volumes to allow detection of low concentration chemical species by GC/MS (the Schott Flash experiments) also failed. With that said, GC/MS was able to identify some organo-sulfur and some other selenium- and sulfur-free volatile compounds in the HS samples.

TICs and mass spectral results obtained for A0LBCd and BW cultures amended with GSH-capped CdSe QDs (1000 ppm) are given in Figs. 14 to 18 on pages 41 to 45. Those amended cultures were incubated anaerobically at 37 °C and HS samples were withdrawn via SPME fiber for GC/MS analysis after 48 h. MeSH, DMDS and DMTS were identified in TICs and verified with the corresponding MS spectra from the NIST database. The temperature program in the chromatographic runs for Figs. 14-16 was without cryogenic oven conditions. The second set of HS samples, including those incorporating the large volume flasks using the enclosure caps, were analyzed with a temperature program that used cryogenic oven temperatures. Mass spectra from these runs allowed the identification of ethyl acetate and heptane, compounds which are invisible to the selective SCD detector, along with MeSH and DMDS. These mass spectra were verified via NIST MS spectra. Propane biosynthesis in bacterial HS with limited oxygen has been reported by Kallio et al. (2014). No other organo-sulfur or -selenium bacterial HS chemicals were identified from the HS sample of bacterial culture via GC/MS.
CHAPTER V

CONCLUSIONS

GSH-Capped CdSe QD Synthesis

GSH capped CdSe QDs with yellowish green fluorescence were synthesized by the hydrothermal method using a 2 h incubation period at 90 ºC. Greenish-yellow fluorescing CdSe QDs were synthesized by the microwave irradiated method. These QDs, synthesized with a 15 s irradiation time with 850 W, were used for the degradation studies.

Fluorescence Spectra

In experiments involving a range of QD concentrations resuspended in sterile LB medium, the highest fluorescence intensity was obtained from 3750 ppm and the lowest from 1000 ppm amended QDs concentrations. Also QD fluorescence spectra revealed unique spectral features which varied with QD concentrations. All prepared CdSe QDs stock concentrations show fluorescence excitation around at 300-450 nm and emission at 500-600 nm as well as resonance Rayleigh and non-linear scattering.

Toxicology of GSH-Capped QDs

Cultures of two Antarctic bacteria and a wild-type E. coli, were successfully grown in sterilized LB media aerobically and anaerobically for toxicological experiments. Growth curves were studied quantitatively to generate specific growth rates. The experiments with GSH-capped CdSe QDs (333/1000/1500 ppm) revealed that the bacteria studied could tolerate and grow in aerobic cultures amended with CdSe QDs at resuspended
concentrations up to 1500 ppm. Moreover, the results highlighted the fact that the GSH-capped QDs were stable in bacterial cultures and did not affect the growth rates of bacteria amended in the log phase of growth.

**Search for Volatile Se-Containing Products of GSH-Capped QDs Degradation**

One way to confirm CdSe decomposition by bacteria is to look for organo-selenium volatile gases in the bacterial headspace of QD-amended cultures (our main research focus). The volatile compounds in the bacterial HS of cultures amended with QDs were analyzed via GC/SCD and GC/MS. Organo-selenium volatiles were present in the HS of cultures amended with *unwashed* CdSe QDs after 48 h incubation at 37 °C, pointing to QD degradation by growing bacteria. However, no volatile organo-selenium chemical species were detected in the HS of cultures amended with CdSe QDs that had first been washed after the synthetic process, removing leftover Se-containing compounds from QD surfaces before they were added to bacterial cultures. Since Se-containing HS compounds were detected in cultures amended with *unwashed* QDs but not in cultures amended with *washed* QDs, we conclude that GSH-capped CdSe QDs are stable under the conditions studied.
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## APPENDIX

### Chemical Abstract Service Registry Numbers

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<th>Compounds</th>
<th>CAS Number</th>
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<td>ethyl alcohol</td>
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<td>L-glutathione reduced</td>
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<td>yeast extract</td>
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VITA

Hemantha K. Siyambalagoda was born on May 25, 1966 in Horana, Sri Lanka to Don Piyasena Siyambalagoda and Gnawatthie Dombagahawattage. He is the first of three children and the first in his family to pursue a degree beyond a Bachelor’s. In 1992, he began working towards his undergraduate degree at University of Sri Jayewardenapura, Sri Lanka. After the completion of four year special degree course, he worked for 12 years in private sector manufacturing companies in Sri Lanka and another four years in the USA after his migration to the United States. Then one day his dream became alive—a higher education in USA—after he received the graduate assistantship from Sam Houston State University, Huntsville, Texas to pursue his Master’s degree in chemistry in September, 2014. He carried out his research under the supervision of Dr. Thomas G. Chasteen in bio-analytical chemistry. During the studies, he successfully competed for the Graduate Student Scholarship in 2014, the Bridge Program Scholarship in 2015 and 2016, and the Graduate Bearkat Grant in 2015 and 2016. His research knowledge was enhanced in working with biochemical collaborators in Chile at The University of Santiago and Andrés Bello University, Santiago, Chile.