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The Effects of Silver Nanoparticles on Wastewater Treatment and Escherichia Coli Growth

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THE EFFECTS OF SILVER NANOPARTICLES ON WASTEWATER TREATMENT AND *ESCHERICHIA COLI* GROWTH

By

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Abstract

Nanomaterials and their increasing use in manufactured products are of great concern to wastewater treatment systems and the environment. Nanosilver has become one of the most popular nanoparticles due to its many applications and relatively low manufacturing costs. It is currently being used for a wide variety of commercial products including medical applications, water purification, antimicrobial uses, paints, coatings, food packaging. Impregnating other materials with silver nanoparticles is a practical way to exploit the germ fighting properties of silver (Nanotechnology 2006). In clothing such as socks, nanosilver may restrict the growth of odor causing bacteria (Benn, Nanoparticle Silver Released into Water from Commercially Available Sock Fabrics 2008). These impregnated socks however have been shown to release large amounts of n-Ag particles when washing. These free nanoparticles easily enter wastewater collection systems.

The microbial inhibitory effects of nanosilver were evaluated by studying the effects *Escherichia coli* (*E. coli*) growth under nanosilver presence. Results showed that *E. coli* bacterial growth was inhibited when nanosilver was introduced. This may have detrimental impacts on aerobic wastewater treatment systems which rely on bacteria to break down organic material. Systems may become inefficient and obsolete with an increasing prevalence of nanosilver in sewage.

1. Introduction

1.1 Nanoscience

NanoTechnology is a growing field in which the understanding and control of matter in the nanometer scale is used to develop new solutions. It involves the manipulation of matter on a near atomic scale to produce new structure, devices, and materials (Morose 2010). Unique physical properties of molecules at the 1-100 nanometer scale make novel applications possible. (EPA, Nanotechnology White Paper. Senior Policy Council. 2007) Nanotechnology has impacted the global market and is currently growing at an exponential rate. The science is estimated to be worth $1 trillion by 2015. (R.D. Handy 2008). Nanotechnology has been developed and used for a variety of applications and due to the potential of this science, there has been a worldwide increase in investment in research and development. (K.A.D. Guzman 2006).

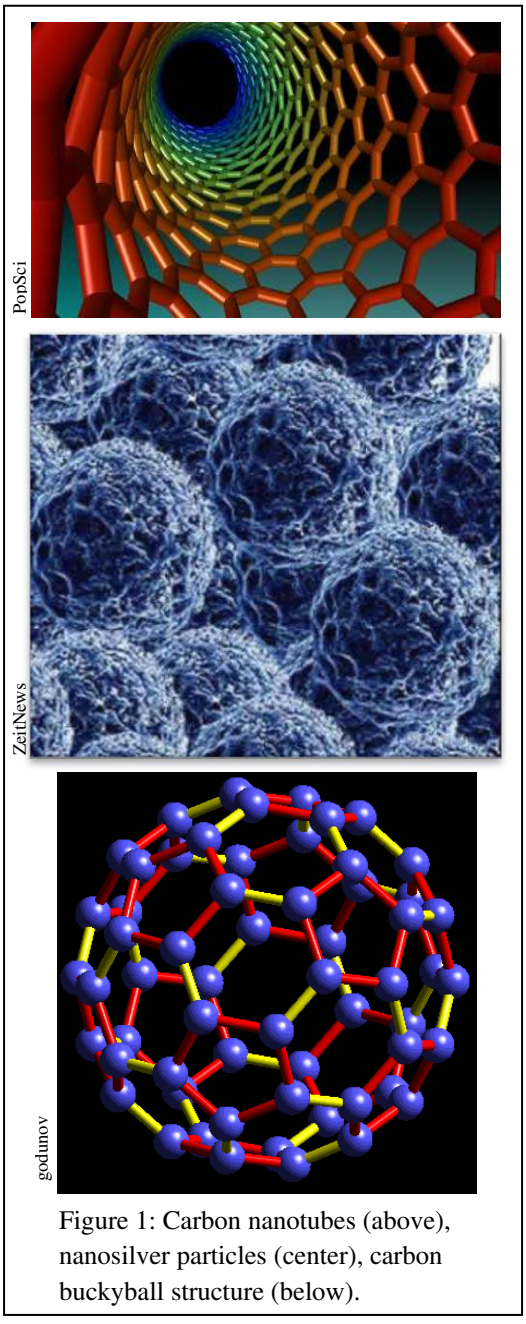

Figure 1: Carbon nanotubes (above), nanosilver particles (center), carbon buckyball structure (below).

Nanomaterials come in an array of forms. The most popular of such include nanoparticles, nanofilms, buckyballs, and carbon nanotubes. Nanoparticles (NPs) are the small-scale substances that have structural components smaller than 1 micrometer in at least one dimension. (Luoma 2008) These particles can be spherical, tubular,

or irregularly shaped. (Nowack 2007) . A nanoparticle is considered nanomaterial when at least two dimensions are between 1 and 100 nanometers. (EPA, Nanotechnology for Site Remediation Fact Sheet 2008). Nanofilms are a film of material in the nanoscale thickness which allow for novel applications. Buckyballs are another type of nanomaterial. They are the roundest and most symmetrical molecule in existence, which is made up of 60 carbon atoms arranged in a series of interlocking hexagons and pentagons, forming a structure that looks similar to a soccer ball (Weber 2009). Carbon nanotubes are composed of a rolled sheet of honeycomb carbon flakes and are the strongest fibers known to exist. Its nano size allows it to be up to 100 times stronger than steel per unit weight. They are extremely strong and have very interesting electrical properties (Crespi n.d.). As you can see, nanomaterials have the potential to change the way humans interact with the environment.

1.2 Applications of Nanotechnology

Nano-sized particles have been present on earth for millions of years and have been used by mankind for thousands of years. An example of such historical use is the production of soot, which is a byproduct of the incomplete combustion of fossil fuels and vegetation. (Nowack 2007)

More than 1000 consumer products that contain NMs are on the market today (Scholars 2007). Nanotechnology has been explored for creating lighter and stronger materials, for remediating contaminated groundwater, for replacing toxic chemicals in various applications, for enhancing solar cell efficiency, and for targeted cancer treatment (Morose 2010). The science is present in environmental remediation, pollution detecting sensors, photovoltaics, medical imaging, and drug delivery. (EPA, Emerging Contaminants-Nanomaterials 2009). Nanotechnology is also present in the electronic, cosmetic, energy, catalytic and material

industries. (Nowack 2007). NMs have been extensively used for rapid or cost-effective cleanup of wastes when compared to current conventional approaches (Satinder K. Brar 2010).

1.3 Implications of Nanotechnology

NMs can be classified as being natural, incidental, and engineered. (EPA, Emerging Contaminants-Nanomaterials 2009). Natural NMs are present everywhere in soils and geologic systems. These natural NPs are also found as aerosols in the atmosphere. Examples of these included soil dust and sea salt (Nowack 2007). Incidental NMs form from engine emissions as a byproduct of combustion (EPA, Emerging Contaminants-Nanomaterials 2009). Engineered NMs are designed with very specific properties which are intentionally produced through certain chemical or physical processes. They may be produced through self-assembly or milling. NMs can then be released into the environment through industrial and environmental applications or improper handling of NMs (EPA, Emerging Contaminants-Nanomaterials 2009).

One estimate for the production of engineered nanomaterials was 2000 tons in 2004. This production is expected to increase to nearly 58,000 tons between 2011-2020. (Maynard 2006) This forecasted dramatic increase in the large scale production and use of engineered NPs makes it likely that increasing human and environmental exposure to NPs will occur with unknown consequences.

Manufactured NMs enter the environment through intentional and unintentional releases. Releases source from atmospheric emissions and solid or liquid waste streams from production facilities. NMs used to remediate contaminated soils can also provide a source of direct intentional NM release into the environment. NPs reaching land have the potential to contaminate soil, migrate into surface and groundwater, and interact with biota. Particles in solid waste, wastewater effluents, direct discharges, or accidental spillages can be transported to

aquatic systems by wind or rainwater runoff. The biggest risks however come from spillages associated with the transportation of manufactured NPs from production facilities to other manufacturing sites, intentional releases for environmental applications, and diffuse releases associated with wear and erosion from general use (Klaine 2009).

1.4 Toxicity

Nanomaterials are of great concern to the environment because of their small size and high catalytic properties (O. H. Choi Vol. 42, No. 12, 2008). NMs are composed of inherently non-biodegradable inorganic chemicals, such as ceramics, metals and metal oxides, and are not expected to biodegrade (Satinder K. Brar 2010). Manufacture, use, and potential release of NMs have preceded evaluation of risk to ecosystems, including humans (Klaine 2009). Sources of NMs will principally originate from the wastewater collection systems in municipalities where large amounts of NPs are released. Increasing use of engineered NP in industrial and household applications will very likely lead to the release of such materials into the environment (Nowack 2007). NPs fate and transport in the environment are largely dependent on material properties such as surface chemistry, particle size, and biological and abiotic processes in varying media (EPA, Emerging Contaminants-Nanomaterials 2009). The toxicity of NPs to organisms has been attributed to their large specific surface area, chemical composition, surface structure, solubility, shape, and charge, as well as aggregation state. These same properties will also control their stability and dispersion in aquatic environments (Choi 2008). The large surface area per unit of vol.ule lends to novel electronic properties relative to conventional chemicals which may also cause some NMs to pose hazards to humans and the environment (Satinder K. Brar 2010). A consistent body of evidence shows that nano-sized particles are taken up by a wide variety of mammalian cell type, area able to cross the cell membrane and become internalized (Nowack

2007). Within the cells, NPs are stored in certain locations and are able to exert a toxic response. Inflammation and fibrosis stress, antioxidant activity and cytotoxicity are observed effects on a cellular level. Ultra –fine soot globules migrate deep into the lungs and carry very toxic, often carcinogenic compounds such and polycyclic aromatic hydrocarbons. Air pollution related illnesses may cause "premature death" (Nowack 2007).

It can be expected that bar screening and other mechanical treatment methods will be ineffective at removing any NPs and thus whatever ends up in wastewater is bound to finally reach the wastewater sludge. When the digested dewatered sludge is sent to landfills or is used as bio-solids for agricultural application, leachability of NPs into groundwater and sub-surface waters is a possibility. Nanopollution in wastewater treatment plants must take into account not just the toxicity of the particles themselves, but also the possible interaction with other environmental contaminants (Satinder K. Brar). Microorganisms are of great environmental importance because they are the foundation of aquatic ecosystems and provide key environmental services ranging from primary productivity to nutrient cycling and waste decomposition (Klaine 2009).

1.5 Legislation of Nanotechnology

The United States Environmental Protection Agency (EPA) has categorized nanomaterials as an emerging contaminant. The EPA defines emerging contaminants as a chemical or material that is characterized by a perceived, potential, or real threat to human health or the environment. (EPA, Emerging Contaminants-Nanomaterials 2009). There is a growing concern about the lack of environmental health and safety data for nanomaterials. Humans can be either directly influenced by NP through exposure to air, soil, or water or indirectly by consuming plants or animals which have accumulated NP. (Nowack 2007)

Due to the lack of understanding in the potentially toxic effects of NP, there is no current law that controls or enforces standards for the production, handling, and disposal of NMs (Bradford Vol. 43, No. 12, 2009). There are also no specific federal standards that regulate NMs based solely on their size (EPA, Emerging Contaminants-Nanomaterials 2009). Many of the currently available nano-products such as those found in cosmetics, drugs and sunscreen products, fall under the Food and Drug Administration's (FDA) regulation (EPA, Emerging Contaminants-Nanomaterials 2009).

However, if NMs enter drinking water or are injected into a well, they may be subject to the Safe Drinking Water Act (SDWA) (EPA, Nanotechnology under the Toxic Substances Control Act 2009). The SDWA is the main federal law that ensures the quality of Americans' drinking water. The law sets drinking water quality standards and oversees water supplier who implement the standards. SDWA was passed in 1974 (USEPA).

2. Nanosilver and Environmental Engineering

2.1 Applications

Nanosilver (n-Ag) are engineered nanomaterials that may come in several forms. N-Ag is primarily produced as colloidal silver, spun silver, nanosilver powder, and polymeric silver (EPA, Emerging Contaminants-Nanomaterials 2009)…N-Ag.is a commercial name for pure de-ionized water with superfine silver in suspension. The size of these n-Ag particles can range between 10 to 200 nm. N-Ag boasts high surface reactivity and strong antimicrobial properties and is produced from elemental silver which is used in many products as bactericide (Nowack 2007).

N-Ag has become one of the most popular NPs due to its many applications and relatively low manufacturing costs. As of 2007, the Project on Emerging Nanotechnologies at

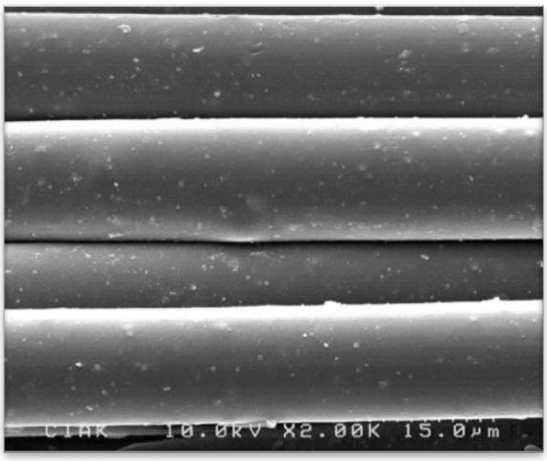

Figure 2: Nanosilver particles attached to polyester yarn (Nano Notice n.d.).

the Woodrow Wilson International Center for Scholars had compiled a list of more than 500 consumer products that claim to include some form of engineered NPs. Of these products, about 20% contain silver NPs (Scholars). It is currently being used for a wide variety of commercial products including medical applications, water purification, antimicrobial uses, paints, coatings, food packaging. To protect from food poisoning, silver particles are now being put in cutting boards, table tops, surface disinfectants, and refrigerators. Manufacturers of clothing articles employ n-Ag as an antimicrobial agent.

2.2 n-Ag in Wastewater

The discharge of nanoparticles from industrial waste or disposal of such materials from commercial and domestic use will inevitably occur with increasing production and enter into wastewater treatment facilities with unknown consequences (Satinder K. Brar 2010). It can be expected that bar screening and other mechanical treatment methods will be ineffective.

Figure 3: n-Ag transport in the environment from manufactured products.

The secondary treatment process is populated by microorganisms where there is a possibility that silver n-Ag may adhere to microbial cell surfaces (Satinder K. Brar 2010). A study conducted by An et al. (2007) demonstrated that the growth of microorganisms was significantly hindered by the silver nanoparticle coating. The potential for nanosilver to adversely affect beneficial bacteria in the environment is a large concern (Satinder K. Brar 2010). When present at higher concentrations, nanosilver may impact the performance of waste treatment processes by inhibiting the growth of microorganisms in secondary treatment processes.

Whatever ends in wastewater is bound to eventually reach the wastewater sludge which is spread on agricultural fields as bio-solids raising issues on the potential leachability of nanoparticles into groundwater and sub-surface waters (Satinder K. Brar 2010). Unextracted n-Ag could pollute the sea, rivers and lakes poisoning a variety of water organisms (Satinder K. Brar 2010). Recent research suggests that some NPs escape from treatment plants and are discharged

into natural water bodies (Satinder K. Brar 2010). Humans can then be either directly influenced through exposure to water or indirectly by consuming plants or animals which have accumulated n-Ag.

2.3 Implications

Impregnating other materials with silver NPs is a practical way to exploit the germ fighting properties of silver (Nanotechnology). In clothing such as socks, n-Ag may restrict the growth of odor causing bacteria (T. W. Benn 2008). These impregnated socks however have been shown to release large amounts of n-Ag particles when washing. These free NPs easily enter wastewater collection systems.

Elemental silver (Ag+) is used in many products as bactericide. (Morones 2005) The highly toxic properties of Ionic silver is believed to be due to its sorption to the negatively charged bacterial cell wall, deactivating cellular enzymes, disrupting membrane permeability, and ultimately leading to cell lysis and death (Ratte, 1999).

The potential for n-Ag to adversely affect beneficial bacteria in the environment, especially in soil and water is of particular concern (An, 2007). In wastewater treatment, where beneficial bacteria that degrade or breakdown organic constituents present in the wastewater, n-Ag with its antimicrobial properties may end up killing the beneficial bacteria which will essentially put a halt to the treatment process (Gellerman, n.d.). Eventually, as n-Ag affects numerous environmental sinks, it may lead to the development of antibiotic resistance among harmful bacteria (Silver 2003). At present little is known about the adverse effects of n-Ag on wastewater treatment and the environment. It is believed however that the inhibitory effect of silver is due to its sorption to negatively charged bacterial cell walls, deactivating cellular enzymes, disrupting membrane permeability, and ultimately leading to cell lysis and death. It is

also believed that silver in the nano scale could be therefore more reactive with its increased catalytic properties and become more toxic than the bulk form (O. D. Choi 2008).

2.4 Case Study: Nanoparticle Silver from Sock Fabrics (Benn 2008)

In clothing such as socks, impregnated n-Ag may restrict the growth of odor-causing bacteria. Six types of socks contained up to a maximum of 1360 ug-Ag/g-sock and leached as much as 650 ug of silver in 500 mL of distilled water. Variable rates of leaching were found throughout the sock types, which lead to believe that the manufacturing process may control the release of silver.

This research has shown that household washing of clothing containing n-Ag may release silver into sewer systems. Most of the n-Ag would then be subject to enter wastewater treatment plants (WWTP) which may have effects on treatment efficiency. N-Ag which is in the WWTP will then sorb onto waste biomass and be introduced into the environment via agricultural land application of wastewater treatment biosolids.

3. Research Objectives

3.1 Nanosilver effect on Water Quality

Research conducted focused on determining what effects n-Ag may pose on current wastewater treatment process. This is of concern due to the use of wastewater treating bacteria present in the aeration stage of treatment. This experiment was conducted in an effort to test whether silver nanoparticles released into wastewater would result in alterations to basic water quality parameters such as biochemical oxygen demand (BOD), turbidity, pH, and conductivity. BOD is used to measure effluent strength in terms of the amount of dissolved oxygen consumed by microorganisms during oxidation of organic components. Generally, higher levels of BOD signify that an effluent has a high microbial count and is thus consuming large quantities of oxygen. BOD5 is determined by taking the difference of the amount of dissolved oxygen present in a sample between a period of five days. Turbidity is another useful water quality gauge. It is quantified by the amount of light able to be transmitted through a sample of water. Impurities such as clay, silt, and organic matter may interfere with the clarity of water. High turbidity levels may characterize water which has originated from erosional runoff, has high level of iron, or has been stagnant for a period of time. pH is the measure of the acidity or alkalinity of a solution. This qualitative measure is taken for determining the limitations of a sample. Conductivity is the measure of the ability of water to pass an electrical current and is affected by inorganic dissolved solids which may be present in a sample.

Figure 4: Obtaining samples from a secondary clarifier at the Thomas P. Smith Water Reclamation Facility, Tallahassee, FL.

3.2 Nanosilver effect on *Escherichia Coli* Growth

After noticing initial differences in dissolved oxygen levels between control and nanosilver rich wastewater, further investigation was done to find evidence of microbial extermination. The objective of this study is to identify the effects of n-Ag on *Escherichia Coli (E. coli)* growth, and to determine at what concentrations n-Ag inhibits E. coli growth. In order to eliminate the possibility of one competing "survivor" microorganism from occupying the niche of an eradicated microorganism, this next study was aimed at observing the effect of nanosilver on a single strand of Escherichia coli in a controlled environment.

To study the biological impact that n-Ag may pose, experiments were conducted to quantify impacts on *E. coli* colonies. *E. coli* is relatively easy to obtain, and amongst other bacteria, work to break down and digest organic materials found in wastewater. Negative implications on the health and longevity of these beneficial bacteria, (imposed by the presence of nanosilver) will ultimately disturb wastewater treatment efficiency throughout the critical aeration stage of treatment. *E. coli* has been chosen as a test subject In order to eliminate the possibility of one competing "survivor" microorganism from occupying the niche of an eradicated microorganism. Microorganisms are convenient test organisms because they grow rapidly and are inexpensive to culture; have a high surface-to-volume ratio, making them sensitive to low concentrations of toxic substances (Klaine 2009). Since the initial *E. coli* seed population is unknown, several control dilutions were conducted in order to obtain a measurable population. These control growths resulted in the maximum quantity of *E. coli* colonies that could be grown from the stock solution without any n-Ag influence.

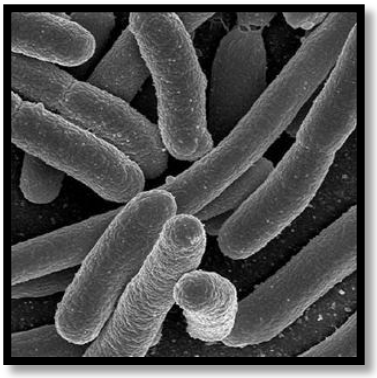

Figure 5: Scanning electron micrograph of E. Coli, grown in culture. Credit: Rocky Mountain Laboratories, NIAID, NIH

4 Materials and Methods

4.1 Nanosilver effect on Water Quality

Nanosilver solution was created by adding 125 mg of nanosilver powder (Aldrich <100 nm 576832-5G) to 250 mL of De-ionized Water (DI-H2O). The solution was mixed by means of magnetic stirring and mechanical agitation. In order to obtain a homogenous sample of n-Ag, the quantities were taken as the solution was kept magnetically stirring. Three dilutions of n-Ag concentrations were prepared and mixed with post secondary clarifier wastewater from the Thomas P. Smith Water Reclamation Facility located in Tallahassee, Florida.

Dilutions of 0 (control), 75 (low concentration), and 2500 (high concentration) ug/L were created by mixing 0, 0.18, and 6 mL respectively of the 0.5 g/L n-Ag solution with 1.2 L of wastewater. After magnetic stirring, each dilution was distributed into four 350 mL BOD bottles. Two of these bottles containing 350 mL each, had an initial dissolved oxygen (DO) reading taken and were then closed with a stopper, sealed with plastic and stored in a dark closet at 23.5 degrees Celsius. These bottles were removed after five days and another DO reading was taken to determine the BOD5. The other two BOD bottles were filled with 250 mL each of the respective dilution and were monitored daily over a five day period for turbidity, pH, and conductivity. All bottles were stored in the same dark environment and four additional BOD bottles were prepared as a control to monitor the experiment quality. Water Quality measurements were taken with Vernier Labquest sensors and probes.

4.2 Nanosilver effect on *Escherichia Coli* Growth

E. coli was grown in de-ionized water and was then treated with the same concentrations as were conducted in initial water quality experiments in order to analyze growth differences between treated and untreated specimens. Several trials were performed to obtain *E. coli* growth results.

Since the initial *E. coli* population was unknown, several control dilutions were attempted to obtain a measurable population. Dilutions were conducted in order to accurately create highly diluted solutions which allowed for the *E. coli* organism concentration to be reduced. The dilutions were required in order to determine a seed concentration that would be suitable for quantifying growth colonies. Once a desired dilution factor was found, *E. coli* was grown with the presence of n-Ag in varying concentrations.

E. coli used throughout the experiments was of stain HB101 (catalog no. 33694) and was acquired from American Type Culture Collection (ATCC). The freeze dried *E. coli* pellet was propagated with molecular biology grade LB Broth (ATCC catalog no. 60-2100).

n-Ag serial dilutions were preformed in order to have a geometric progression of the concentration. *E. coli* colonies were surface-grown by using a membrane filter method (Cellulose Nitrate Filter, Sartorius Stedim, order no. 13906-47-ASN). Cells in the suspension were collected on the membrane through vacuum filtration (Emerson Motor Division model SA55NXGTE-4870). This process was conducted on a sterile clean lab bench. The membrane was then placed on the center of a sterile nutrient agar plate (Sartorius Stedim Nutrient Pads order no. 14068). Colonies on the membrane were incubated (Fisher Scientific Isotemp Incubator model 525D) at a constant temperature of 37 degrees C. Remaining E. coli dilutions were prepared and stored at -80 degrees C in a glycerol mix for future use.

Trial 1 was conducted with three varying concentrations of n-Ag (0, 75, 2500 ug/L) as shown in table 6. Trial 2 depicted in table 7 increased the variances of the concentrations (0, 25, 150, 300, 600,1200 ug/L) with the aim of obtaining a specific lethal concentration. Trial 3 repeated trial 2 with similar n-Ag dilutions and is shown in table 8 and 9 for readings taken at 12 and 24 hours. A fourth trial is planned for early April.

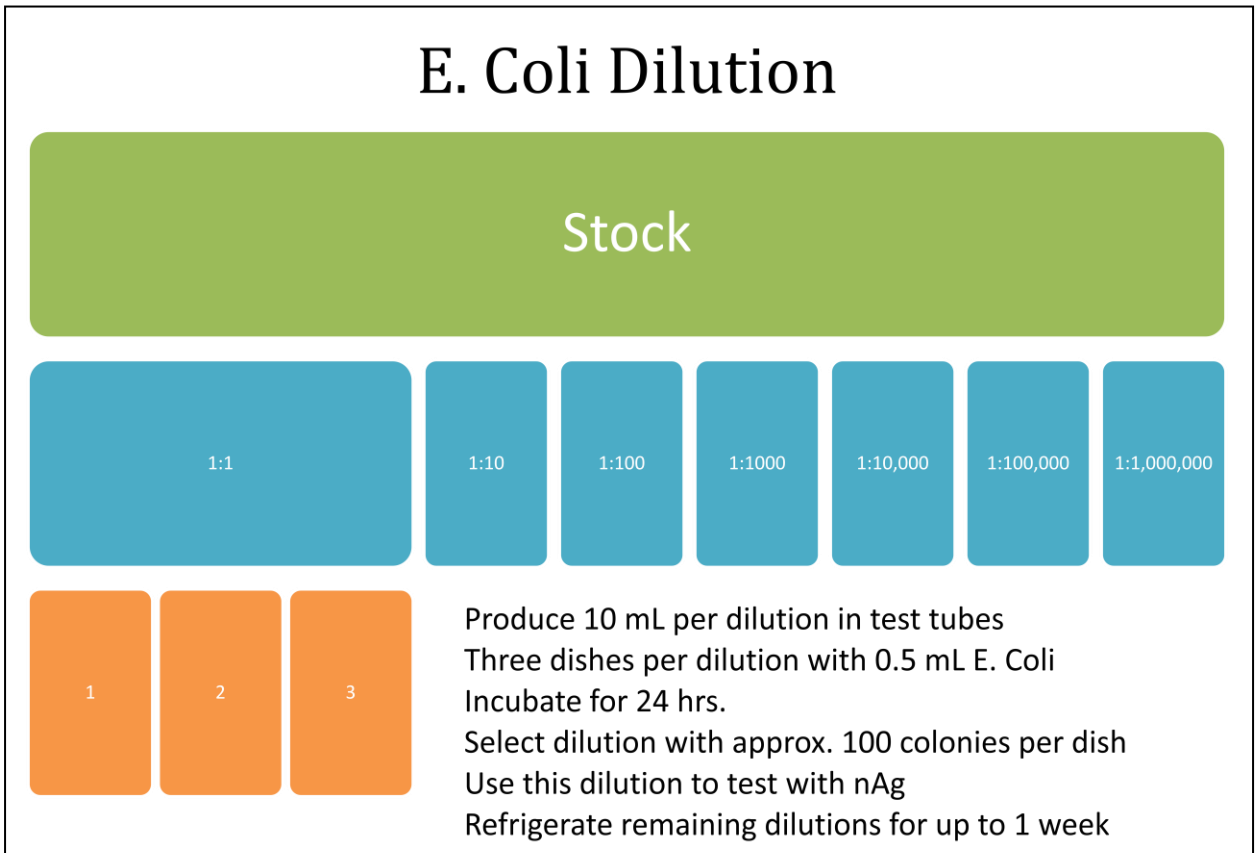

Figure 6: *E. coli* dilution procedures.

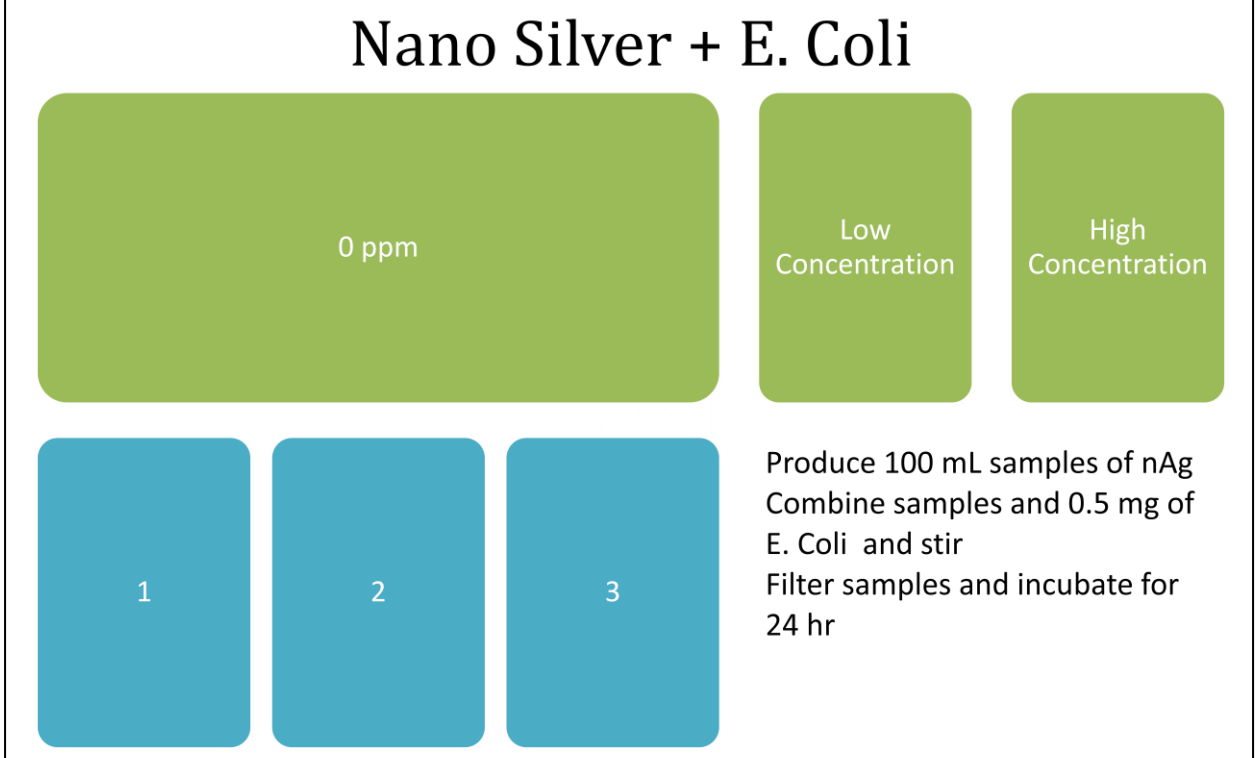

Figure 7: n-Ag introduction into *E. coli* samples.

5 Results

5.1 Nanosilver effect on Water Quality

Experimental results showed insignificant changes to pH, turbidity, conductivity, and BOD5 when n-Ag was introduced to post secondary clarifier effluent. However, it is important to note the differences observed to dissolved oxygen levels after initial introduction of nanosilver to the wastewater. The four samples with n-Ag presence had a higher initial DO level than the two controls which had no n-Ag. Experimental errors could be attributed to the mixing procedure and suspension of n-Ag in de-ionized water. Thorough agitation and magnetic stirring, nanosilver particles would not stay suspended and remained coagulated quickly settling without suspension in water.

Table 1: Summary of dissolved oxygen testing measured over five days for the obtainment of BOD5. Control concentration = 0 ug/L of n-Ag, low concentration = 75 ug/L, high concentration = 2500 ug/L.

Dissolved Oxygen									
			DO (mg/L)						
Sample	**Concentration**	**Test**	0	1	2	3	4	5	**BOD5**
1	Control	BOD5	6.5	-	-	-	-	0.1	6.4
2	Control	BOD5	6.6	-	-	-	-	0.2	6.4
3	Low	BOD5	7.4	-	-	-	-	0.2	7.2
4	Low	BOD5	7.5	-	-	-	-	0.2	7.3
5	High	BOD5	7.5	-	-	-	-	0.4	7.1
6	High	BOD5	7.6	-	-	-	-	0.6	7.0
13	DI Water	BOD5	1.3	-	-	-	-	1.2	0.1
14	DI Water	BOD5	1.2	-	-	-	-	1.1	0.1
15	DI Water	BOD5 Daily	1.8	2.2	2.6	4.3	5.2	5.7	-3.9
16	Control	BOD5 Daily	6.2	5.9	4.1	2.8	1.5	0.5	5.7

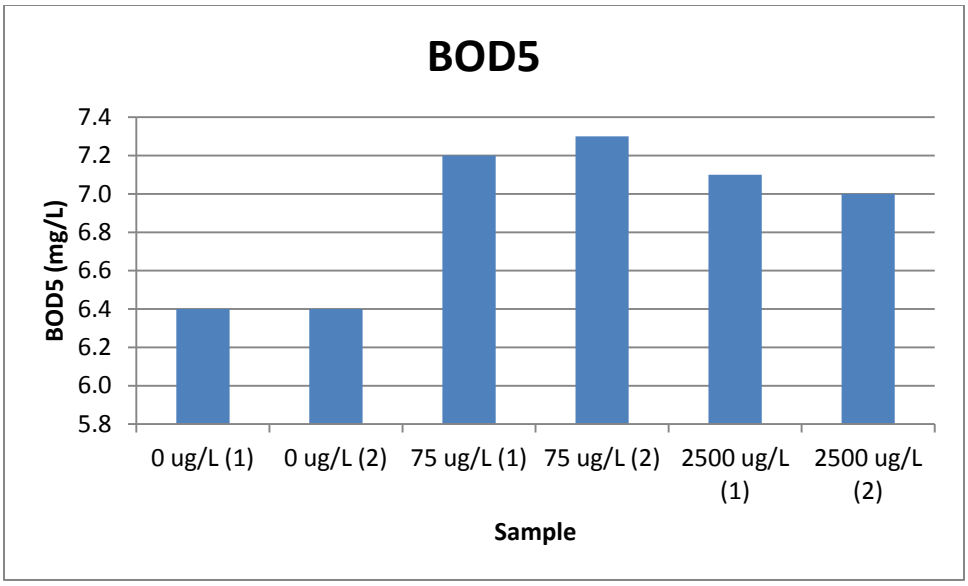

Figure 8: Dissolved oxygen consumption chart of test results for BOD5.

Table 2: Summary of turbidity testing measured over five days. Control concentration = 0 ug/L of n-Ag, low concentration = 75 ug/L, high concentration = 2500 ug/L.

		Turbidity (NTU)					
Sample	**Concentration**	0	1	2	3	4	5
7	Control	2.6	4.2	25.6	-0.3	11.9	12.6
8	Control	7.4	25.8	8.7	6.9	22.5	11.4
9	Low	22.5	13.4	17.7	2.9	10.2	1.8
10	Low	21.8	5.4	12.5	39.9	-5.4	-3.4
11	High	-0.1	2.8	10.2	2.5	21.8	3.6
12	High	-7.5	5.4	11.5	2.3	25.3	7.2

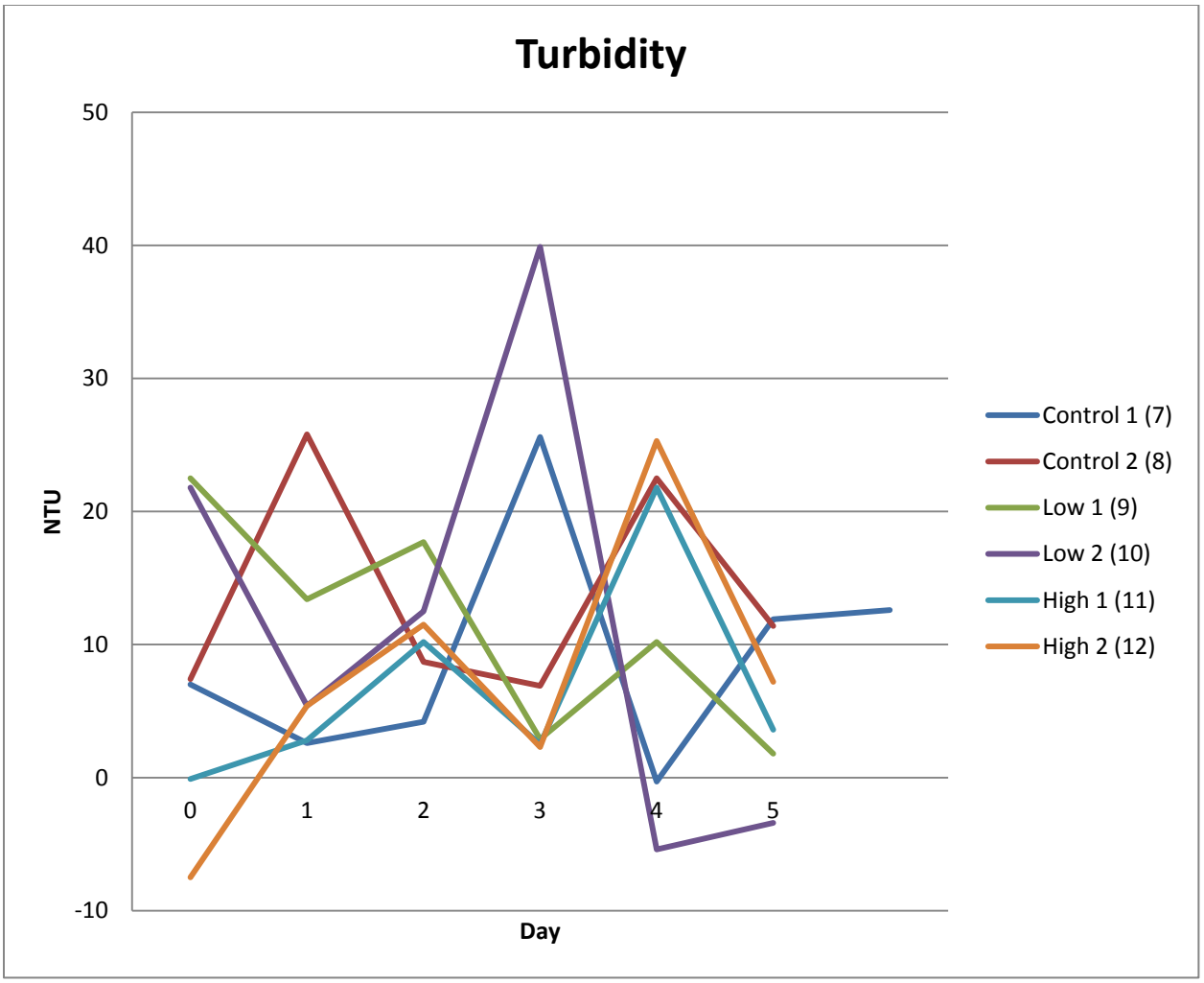

Figure 9: Turbidity plot of test results over a five day period. Control concentration = 0 ug/L of n-Ag, low concentration = 75 ug/L, high concentration = 2500 ug/L.

Table 3: Summary of pH testing measured over five days. Control concentration = 0 ug/L of n-Ag, low concentration = 75 ug/L, high concentration = 2500 ug/L.

		pH					
Sample	**Concentration**	0	1	2	3	4	5
7	Control	8	8	8	8	8	8
8	Control	8	8	8	8	8	8
9	Low	8	8	8	8	8	8
10	Low	8	8	9	9	8	8
11	High	8	8	8	8	8	8
12	High	8	8	8	8	8	8

Table 4: Summary of conductivity testing measured over five days. Control concentration = 0 ug/L of n-Ag, low concentration = 75 ug/L, high concentration = 2500 ug/L.

		Conductivity (uS/cm)					
Sample	**Concentration**	0	1	2	3	4	5
7	Control	1000.4	997.0	997.9	978.2	968.6	967.0
8	Control	997.2	995.8	1002.4	978.6	967.7	968.1
9	Low	989.4	996.8	1003.8	980.8	969.0	970.3
10	Low	987.8	997.8	1004.1	978.8	969.3	972.4
11	High	984.6	995.6	1002.2	979.0	969.1	970.8
12	High	984.0	995.1	1001.2	978.2	967.8	968.9

5.2 Nanosilver effect on *Escherichia Coli* Growth

Nanosilver had apparent growth inhibition on *E. coli* colonies, as fewer colonies were present in n-Ag rich samples. There was an apparent correlation between elevated concentrations of n-Ag and a lower *E. coli* survival rate. Tables 5-8 and figures 12-13 clearly depict results and correlations.

Table 5: Dilution growth of *E. coli* Seed for trials 1 and 2.

Initial *E. coli* Dilution Growth				
Dilution	**ID**	**# of Coliform Colonies Present**	**Avg. (Std. Dev.)**	**Factored # of Colonies**
1:10	A1	46	70 (79.20)	697
	A2	5		
	A3	158		
1:100	B1	56	69 (71.84)	6867
	B2	4		
	B3	146		
1:1000	C1	118	57 (55.47)	56667
	C2	10		
	C3	42		
1:10000	D1	36	104 (83.36)	1040000
	D2	79		
	D3	197		
1:100000	E1	140	93 (76.00)	9266667
	E2	5		
	E3	133		
1:1000000	F1	16	28 (27.07)	2800000000
	F2	9		
	F3	59		
Time in:		6/6/11 1:00		
Time out:		6/6/11 21:50		
Incubation Period:		20.83		
Incubation Temperature:		37 deg C		

Table 6: *E. coli* growth for trial 1 at varying concentrations of n-Ag.

***E. coli* + n-Ag**				
n-Ag Concentration (ug/L)	ID	**# of Coliform Colonies Present**	**Avg. (Std. Dev.)**	**Survival**
0	Z1	76	51.7 (21.08)	
	Z2	40		
	Z3	39		
75	L1	41	23.7 (15.01)	45.81%
	L2	15		
	L3	15		
2500	H1	1	0.3 (0.57)	0.65%
	H2	0		
	H3	0		
E. coli Dilution:		1:100,000		
Time in:		6/6/11 23:10		
Time out:		6/7/11 18:30		
Incubation Period:		19.33		
Incubation Temperature:		37 deg C		

n-Ag Concentration (ug/L)	ID	**# of Coliform Colonies Present**	**Avg. (Std. Dev.)**	**Survival**
0	Z1	>200	111.0 (41.01)	
	Z2	82		
	Z3	140		
75	L1	-		
	L2	-		
	L3	-		
2500	H1	40	20.7 (17.47)	18.62%
	H2	6		
	H3	16		
E. coli Dilution:		1:100,000		
Time in:		6/6/11 23:10		
Time out:		6/8/11 18:58		
Incubation Period:		43.80		
Incubation Temperature:		37 deg C		

Table 7: *E. coli* growth for trial 2 at varying concentrations of n-Ag.

***E. coli* + n-Ag**				
n-Ag Concentration (ug/L)	ID	**# of Coliform Colonies Present**	**Avg. (Std. Dev.)**	**Survival**
0	Z1	381	311.5 (184.89)	
	Z2	55		
	Z3	490		
	Z4			
	Z5	320		
25	25A	117	82.0 (49.50)	26.32%
	25B			
	25C	47		
150	150A	177	102.7 (73.04)	32.96%
	150B	31		
	150C	100		
300	300A	51	76.5 (36.06)	24.56%
	300B			
	300C	102		
600	600A	31	154.5 (174.66)	49.60%
	600B			
	600C	278		
1200	1200A	14	79.0 (91.92)	25.36%
	1200B	144		
	1200C			
E. coli Dilution:		1:100,000		
Time in:		6/8/11 18:58		
Time out:		6/9/11 14:20		
Incubation Period:		19.37		

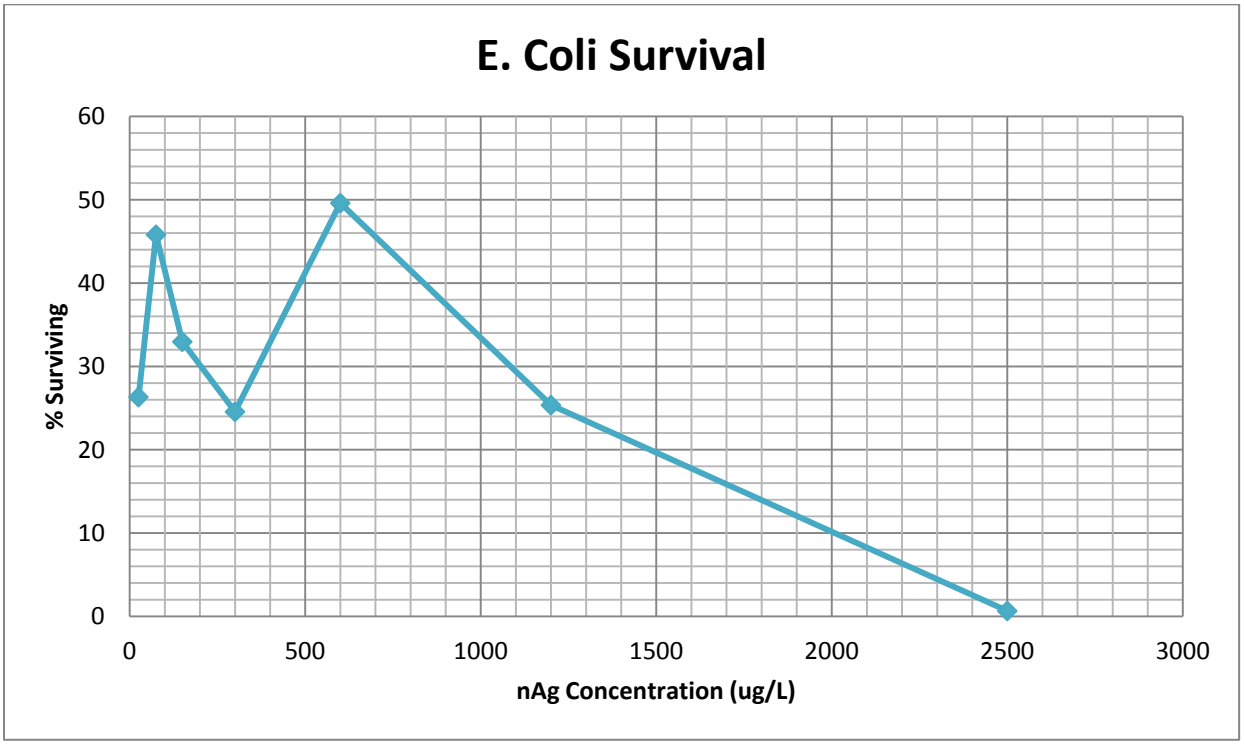

Figure 10: Colony survival for trials 1 and 2 at varying concentrations of n-Ag.

Table 8: *E. coli* growth for trial 3 (12 hours) at varying concentrations of n-Ag.

E. coli + n-Ag				
n-Ag Concentration (ug/L)	ID	**# of Coliform Colonies Present**	**Avg. (Std. Dev.)**	**Survival**
0	Z1	2	79.3 (82.98)	
	Z2	167		
	Z3	69		
25	25A	59	52.7 (47.82)	66.39%
	25B	97		
	25C	2		
75	75A	1	7.3 (6.51)	13.92%
	75B	14		
	75C	7		
150	150A	0	0.0 (0.00)	0.00%
	150B	0		
	150C	0		
300	300A	3	2.3 (1.15)	2.94%
	300B	3		
	300C	1		
600	600A	0	2.0 (3.46)	2.52%
	600B	6		
	600C	0		
E. coli Dilution:		1:100,000		
Time in:		2/7/12 23:30		
Time out:		2/8/12 14:20		
Incubation Period:		14.83		

Table 9: *E. coli* growth for trial 3 (24 hours) at varying concentrations of n-Ag.

E. coli + n-Ag				
n-Ag Concentration (ug/L)	ID	**# of Coliform Colonies Present**	**Avg. (Std. Dev.)**	**Survival**
0	Z1	5	164.0 (154.70)	
	Z2	314		
	Z3	173		
25	25A	46	71.0 (73.75)	43.29%
	25B	154		
	25C	13		
75	75A	2	8.3 (6.51)	11.74%
	75B	15		
	75C	8		
150	150A	0	0.0 (0.00)	0.00%
	150B	0		
	150C	0		
300	300A	15	10.7 (7.51)	6.50%
	300B	15		
	300C	2		
600	600A	1	19.7 (38.20)	11.99%
	600B	58		
	600C	0		
E. coli Dilution:		1:100,000		
Time in:		2/7/12 23:30		
Time out:		2/8/12 23:10		
Incubation Period:		23.67		

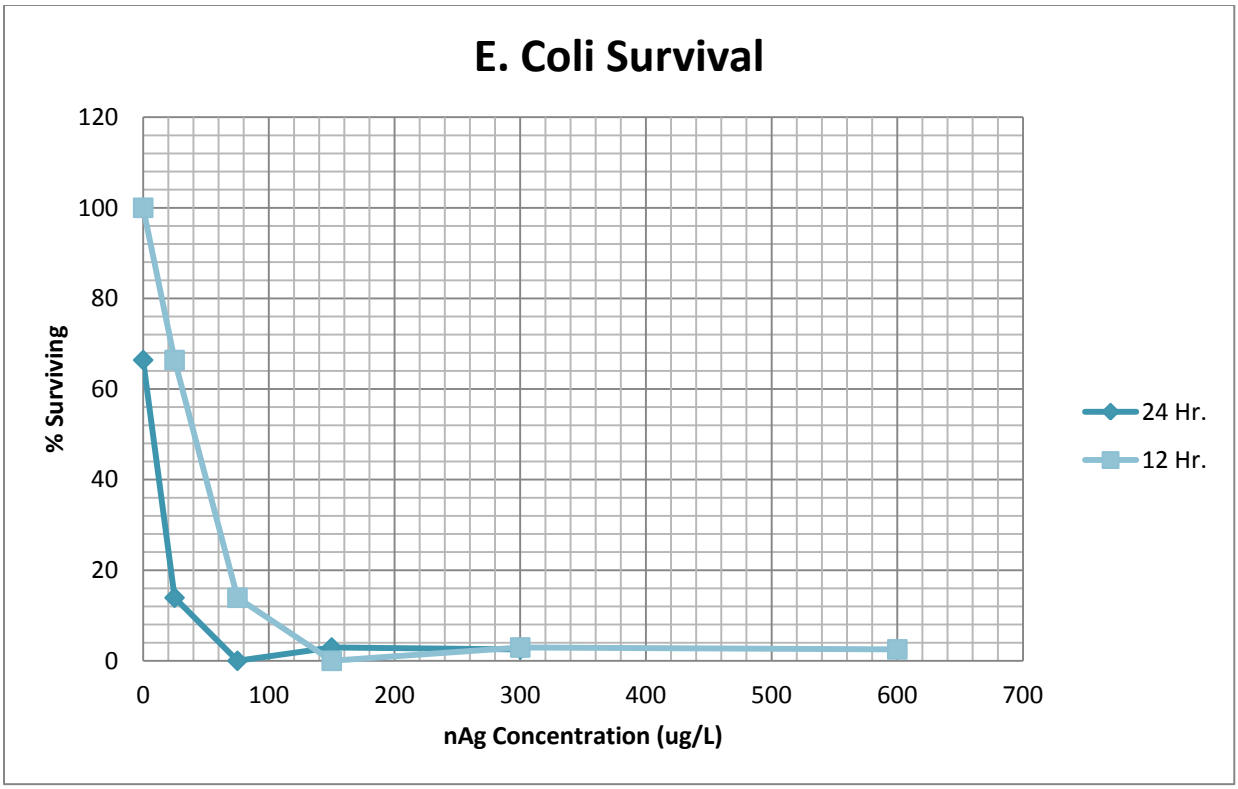

Figure 11: Colony survival for trial 3 at 12 and 48 hours.

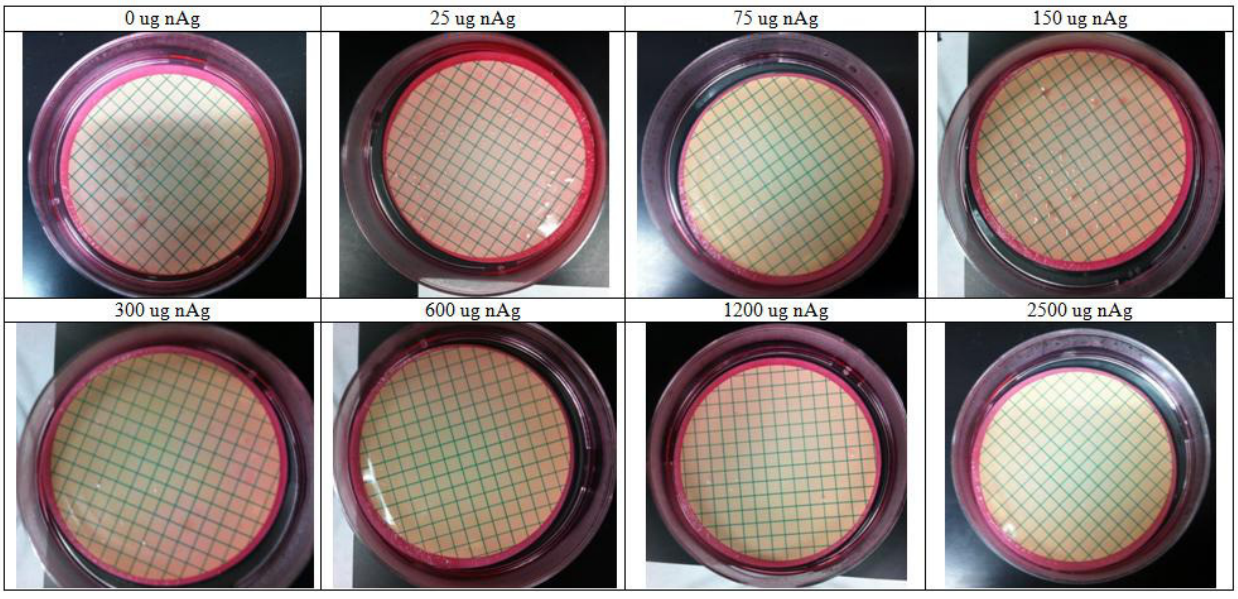

Figure 12: Photographs of incubated *E. coli* plates with varying n-Ag concentrations. Colonies appear as small round rings on the gridded surface.

6 Discussion

6.1 Water Quality

The results obtained from the experiments performed on water quality showed insignificant changes to pH, turbidity, conductivity, and BOD5 when n-Ag was introduced to post secondary clarifier effluent. However, it is important to note the differences observed to dissolved oxygen levels after initial introduction of n-Ag to the wastewater. The four samples with n-Ag presence had a higher initial dissolved oxygen level than the two control bottles which had no n-Ag (Table 1). This change may be a result of an initial organism die off resulting in higher oxygen levels in the n-Ag treated wastewater. The microorganism would have survived in the control batches and thus consumed oxygen. Ultimately most of the oxygen was consumed in all bottles indicating the presence of microorganism survival. The difficulties encountered in keeping the n-Ag particles from agglomerating and suspended in DI water may have contributed in hindering the high biological effectiveness of n-Ag.

Errors could be attributed to the mixing procedure and suspension of n-Ag in deionized water. Thorough agitation and magnetic stirring, n-Ag particles would not stay suspended and remained coagulated quickly settling without suspension in water.

6.2 Nanosilver effect on *Escherichia Coli* Growth

Results from this experiment are useful in evaluating the toxicity of various concentrations of n-Ag on the growth of *E. coli* colonies. The growth and propagation of *E. coli* colonies proved to be tedious and were mostly unsuccessful. Fortunately, several *E. coli* growths were successful allowing n-Ag effects to be studied. The data however was not as concise and predictable as desired. Results from the concentrations of n-Ag sometimes resulted in wide ranges of data and thus the accuracy of those averages is questionable (Table 7). Experimental

procedures were repeated in concurring experiments in order to determine a toxic concentration of n-Ag presence in samples which would lethally disturb *E. coli* colony growth. Preliminary data (Tables 8-9) seems to support previous research where silver NPs have strongly inhibited microbial growth (O. D. Choi 2008) (An 2007). Future experimentation should investigate the role that silver NPs play in its toxicity.

During this thesis defense, it was realized that the HB101 strain of *E. coli* may have not been the best suited for the selected nutrient pads used during filtration and incubation. It was discussed amongst the committee that a strain impregnated with colored dye may have been a better indication for bacterial growth on the nutrient plates. Since there was no coloration on the bacteria, and the growth plates may have not been best suited for *E. coli*, the bacterial growth that appeared, may not have been *E. coli*. Another area of concern in the experiment setup was in the contact time that bacteria had with n-Ag particles. The practice used in this experiment may have only allowed for E. coli to come in contact with the silver particles for the duration of time between n-Ag introduction and sample filtration. The silver particles would have been too small for the membrane filter to collect and would have passed through the filter media while the bacteria remained on the filter surface. It was also noted that the time in which the E. coli and n-Ag particles were in contact may have differed from sample to sample as the total filtration process took several hours to complete, leaving some samples sitting for longer durations of time than others.

7 Conclusion

7.1 Water Quality

Nanosilver samples which showed higher initial dissolved oxygen levels may be a result of an initial organism die off resulting in higher oxygen levels in the nanosilver treated wastewater. The microorganism would have survived in the control batches and thus consumed oxygen. However, ultimately most of the oxygen was consumed in all bottles indicating the presence of microorganism survival. The difficulties encountered in keeping the nanosilver particles from agglomerating and suspended in DI water may have contributed in hindering the high biological effectiveness of nanosilver.

7.2 Nanosilver effect on *Escherichia Coli* Growth

NPs will end up in wastewater and down the line, will end up in soils, sediments, water, finally posing their way into the flora and fauna via food chains. Since wastewater treatment systems can pose as a mainstream in introducing nanoparticles to the environment, it is important to study and understand the effects which nanoparticles such as nanosilver will pose on wastewater treatment systems.

8 Recommendations

In order to fully understand the complete risks associated with the introduction of n-Ag in the environment, it would be helpful to repeat this experiment with a much larger gauge of accuracy. The studies performed in this research focused on determining *E. coli* survival, some other suggestions for future research should also focus on investigating reproductive capacity, and mutation (Klaine 2009).

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