Suresh Gyan Vihar University International Journal of Environment, Science and Technology
Volume 1, Issue 1, 2015, pp.11-15
ISSN:2394-9570

Antimicrobial Activity of Supported Silver and Copper against *E.coli* in Water

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Abstract

The World Health Organization estimates that over one billion people are without access to safe and adequate drinking water sources. An estimated 3.4 million deaths a year are attributable to waterborne diseases. Water borne diseases like cholera, dysentery, typhoid, are caused due to pathogens present in water. Severe bacterial infections and multidrug resistance developed by bacteria is the motive of the study. Antimicrobial effects of copper and silver are studied. Supported material is prepared by wet impregnation method by using copper acetate as the precursor. Antimicrobial activity of the synthesized material is checked against Escherichia coli, gram negative bacteria which are the generally responsible microorganism for water borne disease. The turbidity measurements for the growth determination of E Coli were done by using UV Spectrophotometer at 600nm wavelength. Copper has shown noteworthy growth inhibition of Escherichia coli in water.

Keywords: Water, Escherichia coli, copper, silver, wet impregnation, antimicrobial activity

Introduction

Microbial infections caused by a wide range of pathogenic bacteria, results in mild to serious and life-threatening illnesses, such as blood poisoning, kidney failure, and toxic shock syndrome, which require immediate medic al help and most of the times it is the cause of death in children and the elderly persons. The USEPA recommended that E. coli be used as the indicator micro-organism (indicator of fecal contamination in freshwater recreational areas. Common routes of transmission of micro-organisms are unhygienic food preparation, contaminated greywater or raw sewage, or direct consumption of sewage-contaminated water. Bacteria, with their large populations and fast reproduction time, are able to rapidly develop mechanisms of antibiotic resistance. Organic compounds used for killing micro organisms sometimes are toxic to the human body. Therefore it became essential to discover new phenomenon and to recognize new antimicrobial materials from natural and inorganic substances to build up the next generation drugs or agents to control microbial infections. Many metals have been used from ancient times to prevent spread out of the disease. Among these, Silver and copper found to have better antimicrobial property. The potential of Silver and copper to kill microorganisms have been known from ancient times. Use of these metals cookware and drinking water containers have been used to prevent the spread of disease. Microbiologists and cell culture scientists rely on copper-walled incubators to resist microbial growth. From centuries metals such as silver have been used for treating burns and chronic wounds, and copper has been used to make water potable. Prior to the extensive use of chemotherapeutics in modern health care system, inorganic antimicrobials such as silver and copper were used since ancient times to treat microbial infections [Moghimi 2005].

The health benefits of copper have been known by man since ancient times. The Egyptians used copper to make drinking vessels and water pipes, while others wore copper bracelets to ward off disease. Copper utensils, cookware and drinking water containers have been used to prevent the spread of disease since the early Roman Empire. Copper sink strainers and scourers for pots and pans can help prevent cross-contamination in the kitchen. Silver, a naturally occurring element, is non-toxic, hypoallergenic, does not accumulate in the body to cause harm and is considered safe for the environment. After testing 23 methods of purifying water, NASA has also chosen silver as the purifying agent on the Space Shuttle program. While some natural antibacterial materials, such as zinc and silver, possess greater antibacterial properties as particle size is reduced into the nanometer regime (due to the increased surface to volume ratio of a given mass of particles), the physical structure of a nanoparticle itself and the way in which it interacts with and penetrates into bacteria appears to also provide unique bactericidal mechanisms [Seil and Webster TJ 2012].

It has been known for a long time that silver ions and silver compounds are highly toxic to most bacteria [Slawson et al. 1992, Zhao and Stevens 1998, Spadaro et al. 1974]. A high antimicrobial activity observed for *Staphylococcus aureus*, *Pneumococcus* and *Escherichia coli*, so this material can be a promising antimicrobial biomaterial for implant and reconstructive surgery applications [Díaz et al. 2009]. Products made with AgNPs have been approved by a range of accredited bodies, including the US FDA, US EPA, SIAA of Japan, Korea's Testing and Research Institute for Chemical Industry and FITI Testing and Research Institute [Vivekanandan et al. 2012].

U.S. Environmental Protection Agency, in 2008 registered five copper-alloy products and approved the claim that the metal could kill 99.9% of bacteria within two hours. Clinical trials in the UK, US and Chile have demonstrated that antimicrobial copper touch surfaces reduce contamination by greater than 90% compared to non-copper surfaces. Copper-containing materials for surfaces in the hospital environment may be a valuable adjunct for the prevention of healthcare-associated infections (HCAIs) [Dancer 2009]. *Escherichia coli* O157:H7 is responsible for diseases caused by food contamination. High copper containing alloys greatly reduced the amount of *E. coli* O157:H7 at room (22C) and low temperatures (4C). Stainless steel, the control, had no effect [Noyce et al. 2006]. Cu is coated on single CNT in the state of pure Cu and its oxide. Cu and its oxide play a key role in killing bacteria. The copper-coated CNTs may pave a new way for fabricating artificial heart valves and some other biomedical devices [Tong et al. 2007].

Nanotechnology, the use of materials with dimensions on the atomic or molecular scale, has become increasingly utilized for medical applications and is of great interest as an approach to killing or reducing the activity of numerous microorganisms. While some natural antibacterial materials, such as zinc and silver, possess greater antibacterial properties as particle size is reduced into the nanometer scale (due to the increased surface to volume ratio of a given mass of particles), the physical structure of a nanoparticle itself and the way in which it interacts with and penetrates into bacteria appears to also provide unique bactericidal mechanisms. The larger surface area and reusability of the supported Nano particles of metals lead to synthesis of novel antimicrobial material.

Escherichia coli bacteria are chosen as model microbes for testing anti microbial behavior of the synthesized material. *Escherichia coli* commonly abbreviated *E. coli* is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms. Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls due to food contamination. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2, and by preventing the establishment of pathogenic bacteria within the intestine. *E. coli* and related bacteria constitute about 0.1% of gut flora, and fecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them ideal indicator organisms to test environmental samples for fecal contamination.

Materials and Method

Several methods available for preparation of supported materials are sol gel method, Wet impregnation method, Co-impregnation method and Precipitation deposition method. In wet impregnation method, the support is dipped into an excess quantity of solution containing the precursor(s) of the active phase. This method involves three steps, (i) contacting the support (silica) with the impregnating solution of copper precursor (acetate) for a certain period of time. (ii) Drying the support to remove the imbibed liquid, (iii) Thermal decomposition, followed by activating the material by reduction or other appropriate treatment.

Drying of the synthesized material is done through calcinations. This is a thermal treatment process in presence of air applied to ores and other solid materials to bring about a thermal decomposition, phase transition, or removal of a volatile fraction. The calcination process usually takes place at temperatures below the melting point of the product materials. The process of calcination derives its name from the Latin calcinare (to burn lime) due to its most common application, the decomposition of calcium carbonate to calcium oxide and carbon dioxide, in order to produce cement. The product of calcination is usually referred to in general as "calcine," regardless of the actual minerals undergoing thermal treatment. Calcination is carried out in furnaces or reactors (sometimes referred to as kilns or calciners) of various designs including shaft furnaces, rotary kilns, multiple hearth furnaces, and fluidized bed reactors.

Authors adopted Wet impregnation method using silica as a support and copper acetate solution as the precursor. All materials are purchased from Merck India Ltd. Characterization of the synthesized material was done by XRD, SEM, TEM and Atomic Absorption Spectroscopy to find out % w of copper adsorbed on the support.

The copper-silica composites prepared by wet impregnation method were thermally treated at 500 °C and Samples treated at 500 °C showed the highest surface area and copper exposed on the surface of the silica matrix resulting in a high antimicrobial activity against E. coli. As a result of these processes, the copper species were entrapped inside the vitreous silica matrix.

Results and Discussion

Escherichia coli bacteria were selected as model microbe because they are indicator organism of contamination. Escherichia coli a gram negative microbe, responsible for number of diseases, are members of a large group of bacterial germs that inhabit the intestinal tract of humans and other warm-blooded animals. Authors achieved 20.74% growth inhibition of E coli in 24 hours by using 10mg of synthesized material. There are reports in the literature that show that electrostatic attraction between negatively charged bacterial cells and positively charged nanoparticles is crucial for the activity of nanoparticles as bactericidal materials [Stoimenov et al. 2002, Hamouda and Baker 2000].

Elevated copper levels cause oxidative stress and the generation of hydrogen peroxide. Under these conditions, copper participates in the so-called Fenton-type reaction- a chemical reaction causing oxidative damage to the cell. Excess copper causes a decline in the membrane integrity of microbes, leading to leakage of specific essential cell nutrients, such as potassium and glutamate, and subsequent cell death.

While copper is needed for many protein functions, in an excess situation (as on a copper alloy surface), copper binds to proteins that do not require copper for their function. This inappropriate binding leads to loss-of-function of the protein and/or breakdown of the protein into non-functional portions [Chang et al. 2012]. Distribution water-injured E. coli cells showed a decreased use of oxygen and were likely to depend on fermentation pathways during recovery. This observation leads to the speculation that the cellular damage might be in the respiratory chain [Domek et al. 1984]. Copper kills Staphylococcus Haemolyticus and E coli by damaging cell membrane [Santo et al. 2012].

E. coli cells were damaged by action of silver nanoparticles, showing formation of “pits” in the cell wall of the bacteria, while the silver nanoparticles were found to accumulate in the bacterial membrane. A membrane with such morphology exhibits a significant increase in permeability, resulting in death of the cell [Sondi I and Salopek-Sondi 2004]. Silver nanoparticles of size 10–100 nm have strong bactericidal potential against both Gram-positive and Gram-negative bacteria [Rai et al 2012].

Future Scope

Besides water management, the advantages of antimicrobial properties silver and copper can be utilized in food industries, packaging industries, and textiles, medical and paramedical field and in paint industry.

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