

COMBINED EFFECT OF ULTRASONICATION, UV LIGHT & SILVER NANOPARTICLES ON WASTE WATER TREATMENT

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DECLARATION

I, the undersigned, hereby declare that the research work presented in the M.Tech project entitled "**Combined Effect of Ultrasonication, UV Light & Silver Nanoparticles on Waste Water Treatment** " has been carried out by me under the supervision and guidance of Dr. J. Behari, School of Environmental Sciences, Jawaharlal Nehru University, New Delhi and Dr. K.S. Babu, Assistant Professor, Department of Biotechnology and Environmental Sciences, Thapar University, Patiala.

Further, I declare that no part of this Dissertation has been submitted for a degree or any other qualification of any other university or examining body in India/elsewhere.


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CERTIFICATE

This is to certify that thesis entitled "Combined Effect of Ultrasonication, UV Light & Ag Nanoparticles on Waste Water Treatment" by Ms. Neha Sharma in partial fulfillment of the requirements for the award of Master of Technology (M.Tech) degree in 'Environmental Science and Technology' at Thapar University, Patiala (Deemed University) is an authentic piece of work carried out by her, under our guidance and supervision.

To the best of our knowledge, the matter embodied in this thesis has not been submitted to any other university/institute for award of any degree/diploma.



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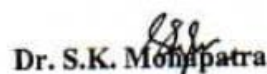
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Abstract

Physical methods of wastewater treatment are useful in water purification and recycling. Combined effect of Ultrasound, UV-C & Silver Nanoparticles offers an attractive proposition in the area of water purification. This work cuts across various chemical, biological and physical boundaries, though, nanotechnology offers the possibility of an efficient removal of pollutants and germs. Ultrasound Reactor Technology (USRT) in a liquid leads to the acoustic cavitation phenomena, such as the formation, growth and collapse of bubbles, accompanied by the generation of local high temperature, pressure and reactive radical species. The possible mechanisms by which cells are rendered inviable during ultrasound irradiation include free-radical attack, including hydroxyl radical attack, and physical disruption of cell membranes. Furthermore, ultrasound irradiation can facilitate the disagglomeration of microorganisms and thus, increase the efficiency of other chemical disinfectants. Present study established the combined effect of US +UV + NP and suggested that it may be more effective than individual. It has been proposed that this may be a useful technique in water purification system. Experiments were conducted using Ultrasonication at three different time intervals (15 min, 30 min & 45 min) followed by three different UV dose (104 mj/cm^2 , 216 mj/cm^2 & 324 mj/cm^2) which was further followed by Ag Nanoparticles treatment at three different doses such as $5 \mu\text{g/ml}$, $10 \mu\text{g/ml}$, $15 \mu\text{g/ml}$ for different treatment time intervals (3 hrs, 6hrs, 9 hrs & 12 hrs). It was found that as the treatment time and dose were increased, better results were obtained in terms of bacterial count i.e. lesser growth of bacteria were seen on petri plate.

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CERTIFICATE

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CHAPTER-1

INTRODUCTION

1.0 Background Information

Water is essential for human life, development and environment, but it is a finite and vulnerable resource which has quantitative limitations and qualitative vulnerability. Water is an important abiotic ecological factor on this planet that maintains the basic biotic processes as well as meteorological phenomenon. As People Action International (PAI, 1997) states, water is the source of life and development on earth. Life is tied to water, air and food, while food is tied to water. Water is a regional resource, but water shortage is becoming a global issue due to increasing population, economic growth and climate change. Development of new sources of water beside its efficient use, together with conservation measures, should be an important component of any country's national water plan. According to a PAI (1999) estimate, there were 31 countries with a total population of 458 million which faced water stress in 1995. More seriously over 2.8 billion people in 48 countries will face water stress by 2025, based on United Nations medium population projections. Gleick (2000) indicates that there are five major drivers demanding a huge expansion of water resources in the 20th century: population growth, industrial development, expansion of irrigated agriculture, massive urbanization and rising standards of living.

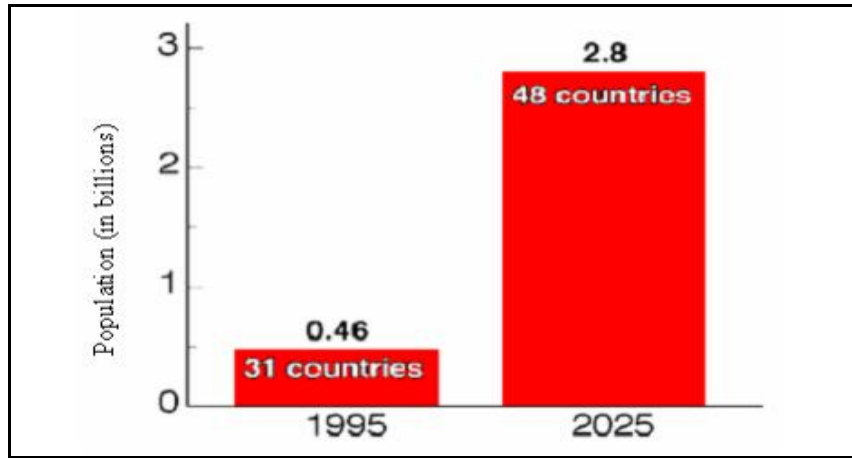


Figure 1. Population in water scarce and water stressed countries , 1995-2025, (Hinrichsen, 1999)

As population increases, the consumption of water in different forms (domestic, industry, irrigation) increases proportionately, which results in the generation of wastewater having multitude of pollutants.

1.1 Waste Water

Waste-water may be in the form of sewage, industrial effluents, urban run off and many others. Waste-water, also spelled waste water, is any water that has been adversely affected in quality by anthropogenic influence. It comprises liquid waste discharged by domestic residences, commercial properties, industry, and/or agriculture and can encompass a wide range of potential contaminants and concentrations. In the most common usage, it refers to the municipal wastewater that contains a broad spectrum of contaminants resulting from the mixing of wastewater from homes, businesses, industrial areas and often storm drains, especially in older sewer systems. Municipal wastewater is usually treated in a combined sewer, sanitary sewer, effluent sewer or septic tank.

Sewage is the subset of waste-water that is contaminated with feces or urine, but is often used to mean any wastewater. Sewage includes domestic, municipal, or industrial liquid waste products disposed of, usually via a pipe or sewer (sanitary or combined). Since last decades, anaerobic digestion is widely used for sewage sludge stabilization, resulting in the

reduction of sludge and the production of biogas. The anaerobic digestion is a slow process, which results in a long residence time in the digestion tank and the requirement of a large tank volume. The adequate treatment and recycling of wastewater before disposal to land field, ocean or, river is essential for sustainable ecological processes in the biosphere. Considering environmental priority, sludge are treated and recycled before safe disposal in environment. The presently available treatment methods are not free from drawbacks, hence efforts are intended to find efficient treatment methods by incorporating ultraviolet (UV), ultrasound (US), ozone (O₃), hydrogen peroxide (H₂O₂) and nanoparticles (NP) as an Advanced Oxidation Process (AOP) (Camel and Bermonda, 1998).

1.1.2 Overview of Waste Water Characterization

To design a treatment process properly, characterization of wastewater is perhaps the most critical step. Wastewater characteristics can be grouped into the following categories:

- Temperature
- pH
- Color and Odor
- Carbonaceous substrates
- Nitrogen
- Phosphorous
- Chlorides
- Total and volatile suspended solids (TSS and VSS)
- Toxic metals and compounds

1.2 Introduction about Ultrasonication, Nanoparticle & UV

Ultrasound (US), Nanoparticle (NP), Ultraviolet light (UV) or in combination (US + NP + UV) offer an attractive proposition in the area of water purification. This work cuts across various chemical, biological and physical boundaries, though, nanotechnology offers the possibility of an efficient removal of pollutants and germs.

1.2.1 Ultrasonication

At present, the wastewater is generally treated by activated sludge process, however, the concentration of the $\text{NH}_3\text{-N}$ and COD in the outlet water cannot meet with the requirement of drainage for environmental protection. It is important to find new efficient treatment processes to remove the $\text{NH}_3\text{-N}$ and these toxically refractory and inert organic compounds. Chemical ultrasonics began in 1927 when Richards and Loomis reported the acceleration of conventional reactions and the reduction-oxidation process by ultrasound. Since then, a number of chemical reactions have been observed in the ultrasonic field. The action of ultrasonic waves in liquids can induce or accelerate a wide variety of chemical reactions. The chemical effects of ultrasound have been explained in terms of reactions occurring inside, at the interface, or at some distance away from cavitation gas bubbles. Chemical ultrasonics began in 1927 when Richards and Loomis reported the acceleration of conventional reactions and the reduction-oxidation process by ultrasound. Since then, a number of chemical reactions have been observed in the ultrasonic field. The action of ultrasonic waves in liquids can induce or accelerate a wide variety of chemical reactions.

The chemical effects of ultrasound have been explained in terms of reactions occurring inside, at the interface, or at some distance away from cavitation gas bubbles. In the interior of a collapsing bubble, extreme but transient conditions are known to exist. It is reported that the temperatures of the cavitation gas bubbles was determined to be up to 5000 K, and the pressures can be increased up to several hundred atmospheres during ultrasonic irradiation. It is expected that such a vigorous effect of ultrasonic irradiation could be applied to decompose some undesired chemicals, especially in the treatment of environmental wastewater. In the few past years, most attention on the application of ultrasonic energy to solve the problems associated with water pollution has focused on decomposing a simple toxic or hazardous organic compound in simulated wastewater, whereas little attention has been paid to decomposing many kinds of pollutant components in environmental wastewater.

1.2.2 NANOTECHNOLOGY-A BETTER TOOL

Nanotechnology is defined as the understanding and control of matter at dimensions of roughly 1-100 nm, where unique physical properties make novel applications possible (EPA, 2007). NPs are therefore considered substances that are less than 100 nm in size in more than one dimension. They can be spherical, tubular, or irregularly shaped and can exist in fused, aggregated or agglomerated forms. Properties of materials of nanometric dimensions are significantly different from those of atoms as well as those of bulk materials. It will offer better built, longer lasting, cleaner, safer, and smarter products for the home, for communications, for medicine, for transportation, for agriculture, and for industry in general. A key understanding of nanotechnology is that it offers not just better products, but a vastly improved manufacturing process. It covers fields from biology to material science, physics to chemistry and can include development in a variety of specialties.

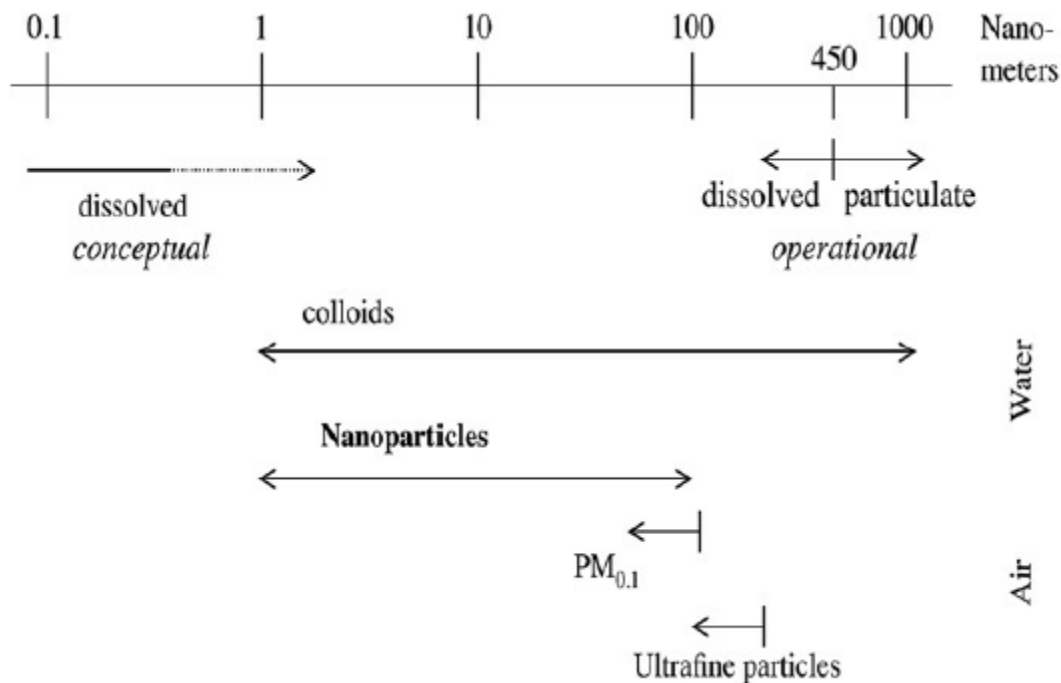


Figure-1.1. Definitions of different size classes relevant for nanoparticles.

Nanoparticles are highly active species. When the particle size is decreased to the nanoscale range, fundamental physical and chemical properties appear to change, often resulting in completely new and different than before physical/chemical properties. One of the most important attributes of all NPs is their high surface area per unit mass. As result of this feature,

the surface of NPs possesses a considerable surface energy (Feldheim and Colby, 2002). As the particle size is reduced, the proportion of atoms found at the surface related to the atoms in the interior of the particle increases and as consequence, the nanoscale particles are more reactive. NPs differ from larger materials in that the number of atoms at the surface and their physical properties are different from those of bulk materials (Poole and Owens, 2003).

Properties associated with the bulk materials are averaged properties, such as density, resistivity and magnetization and the dielectric constant. Critically, however, many properties of these materials change over at the NP scale (Daniel and Astruc, 2004; Niemeyer, 2001). These differences arise from the small size and large number of surface atoms of the particles and related effects. The high surface area to mass ratio of nanoparticles can greatly enhance the adsorption capacities of the sorbent materials. Fundamental electronics, magnetic, optical, chemical, and biological processes are also different at this level. NP can be divided into natural and anthropogenic particles. The particles can be further separated based on their chemical composition into carbon-containing and inorganic NP. Examples of natural NP are fullerenes and CNT of geogenic or pyrogenic origin, biogenic magnetite or atmospheric aerosols (both organic such as organic acids and inorganic such as sea salt). Anthropogenic NP can be either inadvertently formed as a by-product, mostly during combustion, or produced intentionally due to their particular characteristics. In the latter case, they are often referred to as engineered or manufactured NP. Examples of engineered NP are fullerenes and CNT, both pristine and functionalized and metals and metal oxides such as TiO₂ and Ag. Engineered NPs are the main focus of the current research on NP in the environment, but some of them occur also naturally, e.g. as inorganic oxides or fullerenes. In the sections below the different types of natural and engineered NP are presented.

1.2.3 Basic approaches to synthesize nanoparticles

For the production of manufactured NPs, the main objective is not simply to obtain nanoscale materials. For most real world applications, experimental conditions need to be tightly controlled in order to obtain NPs with at least the following characteristics:

- 1) Identical particles in terms of size (a uniform size distribution)
- 2) Identical shape or morphology
- 3) Identical chemical composition and crystal structure (ideally, core and surface

composition must be the same, unless specifically designed for other purposes)

4) Monodispersity (no aggregation)

Table-1.1-Classification of nanoparticles

		Formation		Examples	
Natural	C-containing	Biogenic	Organic colloids	Humic, fulvic acids	
			Organisms	Viruses	
		Geogenic	Soot	Fullerenes	
		Atmospheric	Aerosols	Organic acids	
		Pyrogenic	Soot	CNT	
				Fullerenes	
				Nanoglobules, onion-shaped nanospheres	
		Inorganic	Biogenic	Oxides	Magnetite
			Metals	Ag, Au	
		Geogenic	Oxides	Fe-oxides	
			Clays	Allophane	
		Atmospheric	Aerosols	Sea salt	
Anthropogenic (manufactured, engineered)	C-containing	By-product	Combustion by-products	CNT	
				Nanoglobules, onion-shaped nanospheres	
		Engineered	Soot	Carbon Black	
				Fullerenes	
				Functionalized CNT, fullerenes	
				Polyethyleneglycol (PEG) NP	
		Inorganic	By-product	Combustion by-products	Platinum group metals
			Engineered	Oxides	TiO ₂ , SiO ₂
				Metals	Ag, iron
				Salts	Metal-phosphates
			Aluminosilicates	Zeolites, clays, ceramics	

They can be produced by a huge range of procedures which can be grouped into top down and bottom up strategies (Fig. 1.2). Top-down approaches are defined as those by which NPs or well-organized assemblies are directly generated from bulk materials via the generation of isolated atoms by using various distribution techniques (Niemeyer,2001). The majority of the top-down strategies involve physical methods such as milling or attrition, repeated quenching and photolithography (Gao, 2004).Bottom-up strategies involve molecular components as starting materials linked with chemical reactions, nucleation and growth process to promote the formation of more complex clusters (Gao, 2004; Rotello, 2003).

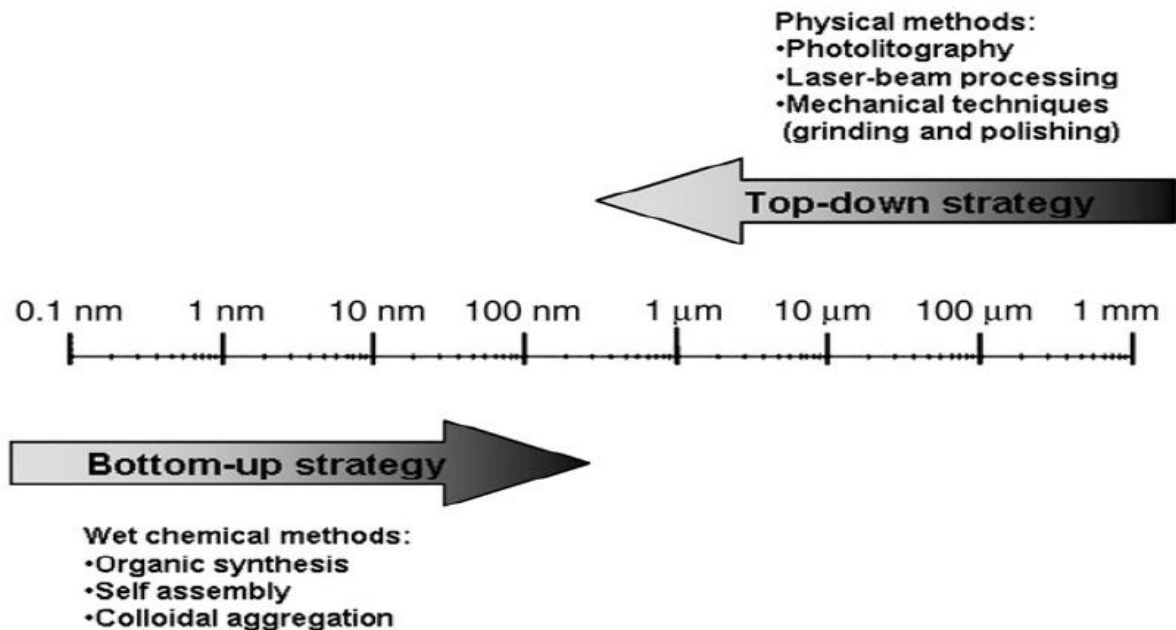


Fig. 1.2 Top-down and bottom-up strategy

1.2.4 Characterization methods for nanoparticles

Characterization of nanomaterial and nanostructure has been largely based on the surface analysis technique and conventional characterization method developed for bulk material. XRD (X-ray diffraction) has been widely used for the determination of crystallinity, crystal structure and lattice constant of nanoparticle, nanowire and thinfilm. SEM (Scanning electron microscope) and TEM (Transmission electron microscope) together with electron diffraction have been commonly used in characterization of nanoparticles; optical spectroscopy is used to determine the size of semiconductor quantum dot. The most common characterization technique for manufactured NPs is high-resolution transmission electron microscopy (HRTEM) (Giersig and Mulvaney, 1993; Hasan et al., (2002), from which an electron micrograph of the NPs can be obtained. However, their dimensions can be determined by a number of Other methods including scanning tunneling microscopy (STM) (Grabar et al., 1997), atomic force microscopy (AFM) (Junno et al., 1998; Li et al., 2003), small-angle X-ray scattering (SAXS) (Nakamura et al., 2003), and X-ray diffraction (Leff et al., 1995). By counting a suitable number of particles (either manually or automatically) size distributions of these NPs gives relevant information on the dispersity of the NPs(Giersig and Mulvaney, 1993).

1.2.5 Applications of nanoparticles

Nanoscience and nanotechnology study have received much attention in the last decade because of our increasing ability to synthesize and manipulate such materials. Today, manufactured NPs are currently used in different areas such as electronics, biomedicine, pharmaceuticals, energy, cosmetics, environmental analysis and remediation, catalysis and material sciences, due to the relative ease with which they can be prepared and manipulated, their generally high reactivity and surface area and the tuneable nature of their optical and other properties (Niemeyer, 2001; Poole and Owen, 2003; Schmid,2004). Because of the potential of this technology there has been a worldwide increase in investment in nanotechnology research and development (Guzman et al., 2006). Data on the current use and production of NP are sparse and often conflicting. One estimate for the production of engineered nanomaterials was 2000 tons in 2004, expected to increase to 58,000 tons in 2011-2020 (Maynard, 2006).

1.2.6 Environmental risks of nanoparticles

The forecasted huge increase in the manufacture and use of NP makes it likely that increasing human and environmental exposure to NP will occur. As a result NP are beginning to come under scrutiny and the discussion about the potential adverse effects of NP has increased steadily in recent years; in fact it has become a top priority in governments, the private sector and the public all over the world (Roco, 2005; Helland et al., 2006; Siegrist et al., 2007). Most attention has thus far been devoted to the toxicology and health implications of NP (e.g. Oberdörster et al., 2005; Kreyling et al.,2006; Lam et al., 2006; Nel et al., 2006; Helland et al., in press), while the behavior of NP in the environment (Biswas and Wu, 2005; Wiesner et al., 2006; Helland et al., in press) and their ecotoxicology (Colvin, 2003; Moore, 2006; Oberdörster et al., 2006a)have been less often reviewed.

Biological and chemical properties of nanoparticles may also differ from the macro form of these substances. This has given rise to some safety concerns for production workers and consumers. Previous reviews (Biswas and Wu, 2005; Handy et al., 2008a, b;Nowack and Bucheli, 2007) have focused on ecotoxicological effects and environmental transport generally. A consistent body of evidence shows that nano-sized particles are taken up by a wide variety of

mammalian cell types is able to cross the cell membrane and become internalized (Lynch et al., 2006; Rothen-Rutishauser et al., 2006; Smart et al., 2006). The uptake on NP is size-dependent (Limbach et al., 2005; Chithrani et al., 2006). Aggregation and size-dependent sedimentation onto the cells or diffusion towards the cell were the main parameters determining uptake (Limbach et al., 2005). The uptake occurs via endocytosis or by phagocytosis in specialized cells. Within the cells NP are stored in certain locations (e.g. inside vesicles, mitochondria) and are able to exert a toxic response. The small particle size, a large surface area and the ability to generate reactive oxygen species play a major role in toxicity of NP (Nelet et al., 2006). Several respiratory and cardiovascular diseases in humans are caused by BC (Avakian et al., 2002; Morawska and Zhang, 2002; Armstrong et al., 2004).

1.3 Objectives of the present study

The problem of inadequate clean water is expected to grow worse in the next coming decades. Finding new ways are important to solve this problem. Sonochemical oxidation is one of the advanced oxidation methods in the area of wastewater and ultrasound provides an appropriate means to change the physical composition of the sample so that “big particles” are transformed into smaller one. Nano-materials represent a promising application in a variety of areas due to their high surface area and reactivity and their ability to become dispersed in aqueous solution. Nano-material usually displays higher reactivity and sorption ability than the same material of normal size & UV light acts as a better disinfection process. Therefore the objectives of the study were:

- **To evaluate the scientific and economic potential of US application as a pre-treatment step in combination with silver nanoparticles and UV to optimize the disinfection process of wastewaters.**
- **To test the combination of Ultrasonic pretreatment, with subsequent UV disinfection & addition of Silver nanoparticles at different doses and treatment time by checking bacterial count.**

CHAPTER-2

REVIEW OF LITERATURE

2.1 Ultrasound

High power ultrasound, operated at low frequencies, is an effective means for disintegration of bacterial cells: first, at low ultrasound doses bacteria flocs can be deagglomerated by mechanical shear stress. When the US dose is increased, ultrasound cavitation can destroy cell walls. This effect is lethal to the microorganisms.

2.1.2 Characteristics of ultrasound

Ultrasound includes a wide range of frequencies between 20 kHz and 10 MHz (Laborde et al., 1998a; Laborde et al., 1998b). There are two types of acoustic cavitation: transient and stable. Ultrasonic cleaning frequencies, typically between 20 kHz and 350 kHz, transform low energy/density sound waves into high-energy/density collapsing bubbles, producing transient acoustic cavitation. Transient acoustic cavitation can cause damaging surface erosion in more sensitive substrates. However, megasonic frequencies, 700–1000 kHz, produce stable acoustic cavitation bubbles, which have less time to grow, and are smaller, resulting in a less vigorous collapse than in transient cavitation. Acoustic energy is supplied to a liquid, gas bubbles are formed and grow by absorbing gas and vapour from the liquid (Laborde et al., 1998a; Laborde et al., 1998b; Porten langer, 1999; Neis,2000). The most commonly used frequencies for industrial cleaning are those between 20 kHz and 50 kHz. Frequencies above 50 kHz are more commonly used for high precision cleaning, removal of small particles and delicate parts (Suslick and Price, 1999;Gelate et al., 2000). Ultrasound wavelengths measuring roughly 10 to 10⁻³ cm are not comparable to molecular dimensions. Because of this mismatch, the chemical effects of ultrasound cannot result from a direct interaction of sound with molecular species (Suslick, 1994; Gong and Hart, 1998).

2.1.3 Generation (Ensminger, 1973)

Ultrasonic energy is generated and detected by devices called transducers. By definition, transducer is a device that is actuated by power from one system to another one. In ultrasonics, the most typical conversions are electrical to ultrasonic energy (transmitters) or ultrasonic to electrical energy (receivers). Transducers most often used for generating ultrasonics are piezoelectric, magnetostrictive, electromagnetic, pneumatic and mechanical devices. Piezoelectric transducers utilize piezoelectric components such as plates or other suitable configuration, which generate a charge on, preferred surfaces under the influence of stresses, or which change dimensions when they are subjected to an electric field. Magnetostrictive transducer utilizes materials, which change dimensions under the influence of a magnetic field. Electromagnetic transducers make use of the attractive forces of electromagnets to generate vibrations. Mechanical transducers are devices, which are actuated mechanically. Their applications are more correctly classed as macrosonic. They are used to obtain high-amplitude, high-intensity vibration at low sonic frequencies.

2.1.4 Source

The most intensive source of ultrasound generally used in the chemical laboratory is the direct immersion ultrasound horn (having piezoelectric material PZT), which has been adapted for inert atmosphere work or for moderate pressures (<10 atm). A variety of sizes of power supplies and titanium horn is available, thus allowing flexibility in sample size. The acoustic intensity is easily and reproducibly variable. The acoustic frequency is well controlled. Metals having high tensile strength and low reactivity is resistant to corrosive media so used in both homogenous and heterogeneous sonochemistry (Babish and Lisk, 1981).

2.1.5 Cavitation

The main mechanism of sonication is based on the cavitation phenomenon which includes the whole procedure of creation, expansion and collapsing of microbubbles throughout liquid phase when negative pressure is applied to the medium during sonication.

Microbubble collapsing typically produces high temperature and pressure condition locally throughout the liquid phase; however the whole liquid mass stays at ambient conditions. This collapsing of microbubbles can produce other physical and chemical changes. These can

generate a great shearing force inside the liquid bulk which can mix and break particles. Figures 3.1 and 3.2 typically indicate the procedures of microbubble collapsing due to cavitation. Sonochemical reactions are recognized as such chemical reactions in which the violent collapse of cavitation bubbles created by intense sonication generates oxidants such as hydroxyl radicals and hydrogen peroxide in liquid bulk.

2.1.6 Mechanism of ultrasonic action: sonochemistry

Ultrasound Reactor Technology (USRT) in a liquid leads to the acoustic cavitation phenomena, such as the formation, growth and collapse of bubbles, accompanied by the generation of local high temperature, pressure and reactive radical species (Neppiras, 1980; Hua and Hoffmann, 1997; Kalumuk, 2003; Hadi et al., 2007). Acoustic cavitation may affect a number of mechanical, acoustic, chemical and biological changes in a liquid (Lauterborn and Ohl, 1997; Laborde et al., 1998a). The possible mechanisms by which cells are rendered inviable during ultrasound irradiation include free-radical attack, including hydroxyl radical attack, and physical disruption of cell membranes (Scherba et al., 1991; Phull et al., 1997). Once the cell membrane is sheared, chemical oxidants can enter the cell and attack internal structures, or vital structures can be released from the cell, and degraded in solution. Furthermore, ultrasound irradiation can facilitate the disagglomeration of microorganisms and thus, increase the efficiency of other chemical disinfectants (Scherba et al., 1991; Petrier, 1992; Phullet al., 1997; Hua and Thompson, 2000). Rechards and Loomis in 1927 first reported the chemical effects of high-power ultrasound (Rechards and Loomis, 1927). Two types of chemical reaction are described:

- 1 The acceleration of conventional reactions by ultrasound
- 2 Redox processes in aqueous solution.

In a liquid medium, the effect of ultrasound is produced due to phenomenon called cavitation (Suslick, 1988). Cavitation as a phenomenon was first reported in 1895 by John Thorny croft and Sidney Barnaby. Since 1945, an increasing understanding of the phenomenon of cavitation has developed coupled with significant developments in electronic circuitry and transducers (i.e. devices which convert electrical to mechanical signals and vice versa). As a result of this there has been a rapid expansion in the application of power ultrasound to chemical processes, a subject that is known as ‘Sonochemistry’ (Suslick, 1994; Gelate et al., 2000). Flym (1964),

Atchley (1988), Lickiss and McGrath (1996) reported that, during the negative cycle of the wave, the distance between the molecules of the liquid will vary (oscillate) about a mean position. If the distance between the molecules exceeds the critical molecular distance $[(R)$ e.g. for water R is 10–8 cm], then the liquid will break down and voids will be created, i.e. formation of cavitation bubbles.

During the positive cycle of the wave, the bubbles grow in size due to the positive acoustic pressure and then finally collapse, leading to the formation of new nuclei for the next cavitation. In water implosion and fragmentation of the bubble which collapses the centre of high energy phenomena; temperature, pressure and electrical discharges giving rise to direct destruction of macromolecule of solute and the sonolysis of water to form H and OH radicals (Sehgal and Wang, 1981; Suslick, 1988; Edwin et al., 1990; Henglein and Gutierrez, 1990). OH· radical reacts with another OH· (hydroxyl) radical to form hydrogen peroxide. Both H₂O₂ and OH· are strong oxidizing species (Bechtels and Wagner, 1999). On sonolysis of water (aqueous solution), hydroxyl radical (OH·) and hydrogen atom form as per reactions shown in Figure 1. It is very important to induce acoustic cavitations, which produces pressure waves responsible for generating gas and vapour bubbles that grows and collapse violently at high velocity. Bubble of cavitation may function as a micro reactor. The cavitation bubbles are vapour filled and surrounded by a liquid hydrophobic boundary layer. Therefore, preferably volatile and hydrophobic substances are accumulated in the cavitation bubbles where this will attack with their layer of pyrolytic centre (temperature: ~ 3000–5000 K; pressure: ~ 1000 atm) or radical reactions and interfacial region (temperature: ~ 300–2000 K) (Figure 1). Some of the radicals escape from the vapour and reach the liquid boundary layer and pass on into the bulk solution where reactions with hydrophilic substances take place and organic compounds are degraded. OH· radical escape out of bubble and reacts rapidly with compound in solution and get oxidized (Makino et al., 1983).

2.1.7 Sonochemical factors

A number of factors influence sonochemical activity. These include frequency (Cum et al., 1992; Petrier et al., 1992; Entezari and Kruus, 1994) sparge gas (Hua and Michael, 1997; Beckett and Hua, 2001) ultrasonic power (Kimura et al., 1996), reactor pressure and solution

temperature (Sehgal et al., 1980; Didenko et al., 1993). Individually, each of these factors may considerably affect the ultrasonic process and when combined may enhance ultrasonic effects (Suslick, 1988). Frequency is a significant factor in determining optimal reaction conditions. The optimum frequency is substrate specific (Petrier and Francony, 1997). Commonly accepted indicators for the selection of the optimum frequency are not available yet because of limited understanding about the influence of frequency on acoustic cavitation and sonochemistry (Beckett and Hua, 2001).

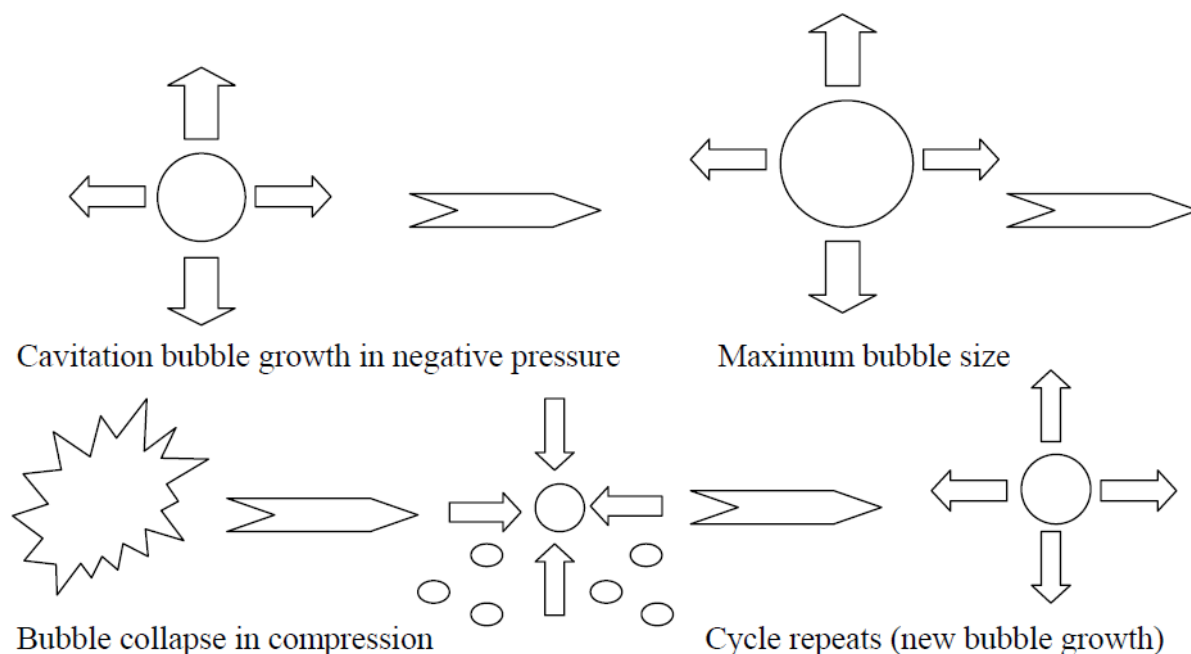


Figure 2.1. Microbubbles collapsing procedures due to cavitation

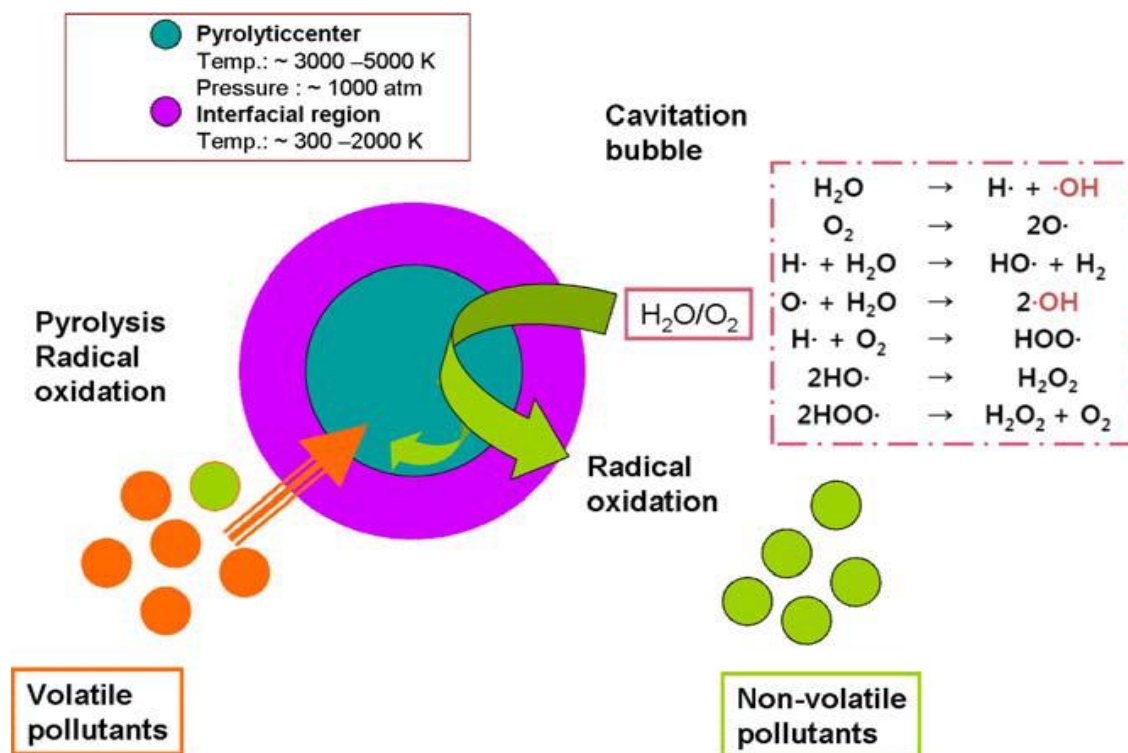
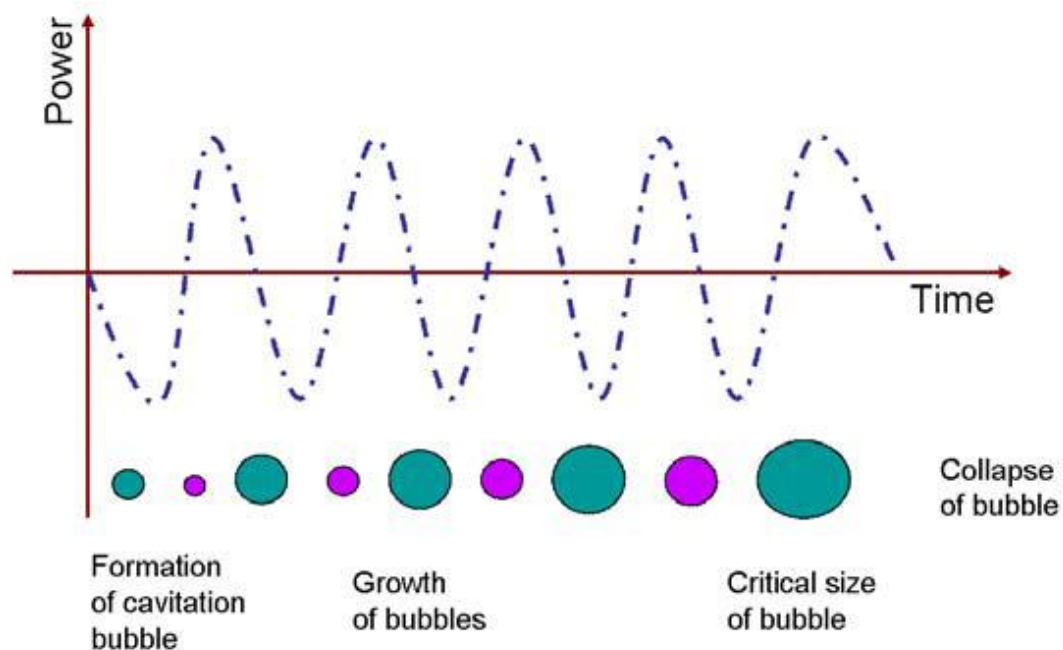


Figure 2.2. Reaction mechanism of cavitations in ultrasonic field

2.1.8 ROS formation during Ultrasonication

Reactive oxygen species are chemical species with one unpaired electron derived from molecular oxygen. Molecular oxygen in the ground state contains two unpaired electrons known as a triplet state having same spin of electron. The excitation of electron by stress, ultrasound, ultraviolet, nanoparticle, electromagnetic field, etc., changes the spin of electron resulting in oxidant activity. Free radicals are highly reactive molecules with a very short half-life. However, in such time, they may cause damage to the cells. The balance between production and neutralization of ROS is maintained by concert action of enzymatic and non-enzymatic defence systems. ROS levels can increase dramatically, which may cause damage to cell structures and react with various biochemical reactions. When unbalanced, it may lead to oxidation of polyunsaturated fatty acids in lipids, amino acids in proteins and damage to DNA. As a potential mechanism for disinfecting agents in the sonochemical disinfection, increasing attention has been given to Reactive Oxygen Species (ROS), such as OH, O₃, H₂O₂ and •O₂, which is produced due to cavitation water bubbles during ultrasonic disintegration of waste sample. The high oxidation potential of ROS makes them more effective disinfectants for all kinds of microorganisms. Although a number of studies have been conducted to investigate the role of ROS in sonochemical disintegration of waste water, most of these focused on the possibility that ROS might enhance the inactivation efficiency of microorganisms but failing to provide experimental results which are relevant to the direct role of ROS. In addition, determining the exact mechanism by which ROS enhance the disintegration process is complicated by the presence of the active free radicals generated by cavitation bubbles present in ultrasonic treated sample. Furthermore, it is not as clear to which species of ROS is more significant for disruption of microorganisms when the reaction conditions are varied.

2.2 Silver Nanoparticles for Waste Water treatment

Among the inorganic antibacterial agents, silver (Ag) has been known most extensively since ancient times to fight infections and control spoilage. The antibacterial and antiviral actions of Ag, Ag⁺ and Ag compounds have been thoroughly investigated. It is well known that silver ion and silver-based compounds are highly toxic to microorganisms, showing strong biocidal effect against as many as 16 species of bacteria, including *Escherichia coli*. Silver nanoparticles are known to be good antibiotic agents. However, Ag⁺ ions or salts have only limited usefulness

as antimicrobial agents for several reasons: interfering effects of salts and the antimicrobial mechanism of continuous release of enough concentration of Ag ion from the metal form. In contrast, these kinds of limitation can be overcome using Ag nanoparticles. Nanoparticles are highly reactive species because of large surface area.

Ag nanoparticles are attractive as these are non-toxic to the human body at low concentration and have broad-spectrum antibacterial nature. Silver nanoparticle has the capability to kill living contaminants present in wastewater as well as permanently bind to the filter membrane. Size and stability are important factors for the biocidal nature of Ag nanoparticles.

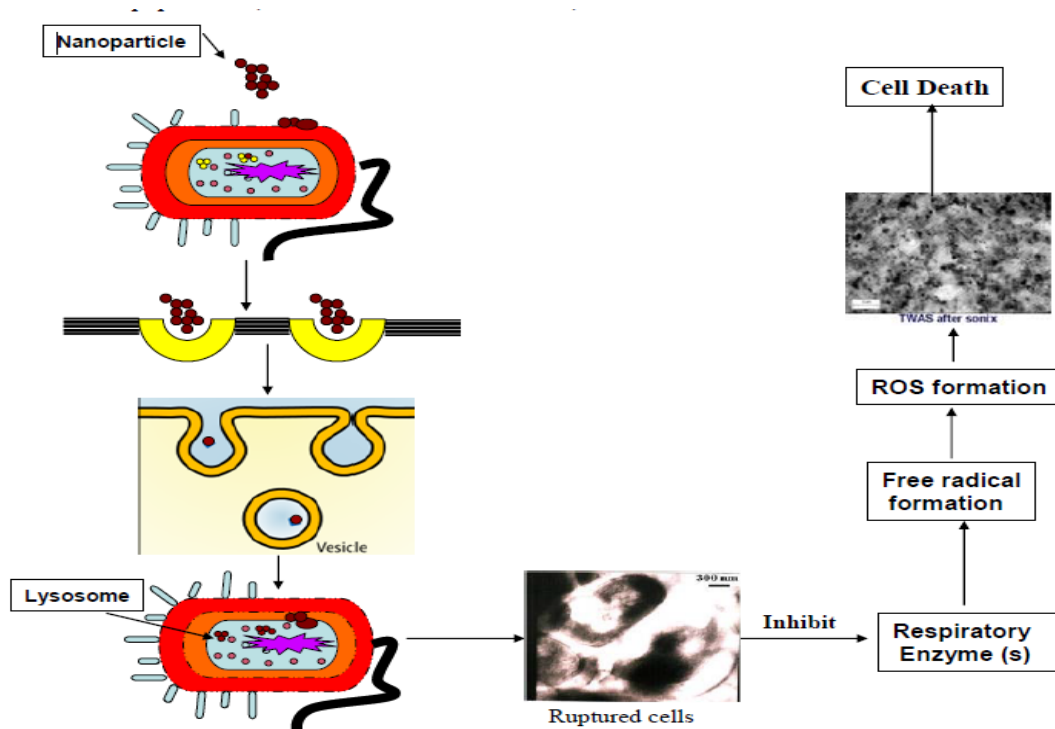


Figure 2.3. Interaction mechanism of reactive oxygen species with nanoparticle on bacterial population.

2.1 UV Application (UV disinfection of WasteWater)

Approximately 10% of the total sunlight reaches to the earth consists of UV light. The use of UV light for the disinfection of wastewater has been started at the beginning of the 20th century. The first performance of UV disinfection for drinking water was started in Marseille, France in 1906-1909 in large scale and it was used for disinfection of ground water in another city in France, Rouen. During the World War I the improvement of UV disinfection of wastewater has been stopped for a while. In the United States, the implementation of UV disinfection started in 1916 in Henderson, Kentucky. All the UV disinfection implementations for wastewater were stopped during 1930s and the chlorine disinfection became the preferable method again due to its lower costs and easier way to implement. In 1950s UV disinfection of wastewater improved again. Nowadays, in Europe, there are more than 3000 UV disinfection instruments which are being used in different types of water disinfection like supplying municipal potable water and ultra pure water for pharmaceuticals and medical industries. In the United States and Canada, the wide implementation of UV disinfection of water is driven by increase in need for wastewater treatment and environmental concerns over disinfection by-products.

In practice, UV light can be generated by an electrical discharge through the mercury vapor lamps. UV light can be absorbed by the microorganisms' nucleic acid (DNA and RNA) and subsequently by destroying their molecular structures and prevent their reproducibility. UV can inactivate bacteria, viruses and spores. UV light has also the ability to produce hydroxyl radicals. Hydroxyl radicals are strong oxidants that can inactivate microorganisms. The efficiency of UV disinfection depends on the concentration of microorganisms, particulate size, UV dose absorbed by the microorganisms and UV transmission through wastewater. UV applications efficiency is limited for samples with high concentrations of suspended matter. Recent studies have shown that large particles (bigger than 50 μm in diameter) are hard to penetrate so that the required UV demand is raised drastically. Therefore, it is common practice to install and filters (e.g. rapid sand filters) to reduce particulate matter prior to the UV step. Rapid sand filters are expensive in construction and maintenance. They are well known in potable water production, but when it comes to wastewater treatment there are many drawbacks

(e.g. clogging, algae growth, backwashing). Another attempt to bring down the size of agglomerates of particles is the application of ultrasound (US).

2.2.2 UV light classification

Regarding to the spectrum of electromagnetic radiation ultraviolet appears with the wavelengths ranging from 100-400 nm. However, the region between 200-300 nm has the best ability to stop the reproducibility of microbial particles. UV light, specifically around the wavelength of 254 nm can penetrate through the cell wall and get absorbed by cellular material and can prevent the replication of the cells or kill the cells. UV light is divided into three subgroups regarding to their wavelengths, the table below has shown these three subgroups:

Table 2.1. UV Light subgroups

Type	Range	Comment
UV-A	From 400 to 315 nm	Between 400 and 300 nm, called near UV
UV-B	From 315 to 280 nm	Called medium UV
UV-C	From 280 to 200 nm	Range to be considered in water disinfection

2.2.3 UV dosage

The UV irradiation energy reaches to surface water with the unit of mJ/cm^2 is called UV dose. It is essential in UV disinfection of wastewater to measure the amount of UV energy that is delivered to the disinfection medium.. The microbial inactivation degree depends on the UV dosage received by the microorganism defined by:

$$\text{UV Dose (mj/cm}^2\text{)} = I \times t$$

Where, I is the average UV light irradiation intensity and t is the UV light irradiation exposure

time. The UV light intensity is reduced when it passes through the media like water and has to be corrected for UV transmittance of wastewater. UV transmittance indicates the ease of passing UV light through water and water absorbing tendency.

2.2.4 UV Dose Response Curve (UV-DRC)

UV dose response curve is the plot of surviving colony forming units (CFUs) versus UV dose. UV dose response curve usually is presented in a semi-logarithmic form and consists of two parts: a linear initial slope at low UV doses corresponding to an exponential decay in CFUs, followed by a near-plateau region at high UV doses known as the tailing region .

2.2.5 Factors Influencing UV Disinfection

As mentioned before, microorganisms' concentration, particulate size, absorbed UV dose by the microorganisms and UV transmission in the water affect the efficiency of UV disinfection. Following Table indicates the major parameters affecting UV disinfection.

Table 2.2. Key parameters affecting UV disinfection and their typical values

Parameters	Typical values
Percent transmittance (T) or absorbance	35-65
Total suspended solids (TSS) (mg/l)	5-10
Particle size (µm)	10-40
Iron (mg/l)	Less than 0.3
Hardness (mg/l)	Less than 300
Flow rate or hydraulics	-

The key wastewater parameter in UV disinfection is the UV transmittance or UVT. UVT indicates the ease of passing UV light through the solution and furthermore the UV demands for the different effluents . Since 254 nm is the most effective wavelength for microbial inactivating, UV transmittance is usually measured by an UV spectrometer operating at the wavelength of 254 nm . In this wavelength the UV transmittance percentage relating to the distilled water is set at 100%. A low UV transmittance shows that a lesser amount of the UV light can reach the targeted microorganisms, and hence lower disinfection efficiency is obtained. can reach the targeted

microorganisms, and hence lower disinfection efficiency is obtained. Dissolved particles through water can affect the UV transmittance adversely due to their UV absorption characteristics. The existence of suspended particles and dissolved chemical compounds which can absorb UV light such as iron can affect the UV light transmittance. The particles can decrease the efficiency of UV disinfection by absorbing or scattering the UV light, or protecting the microorganisms from exposure to UV light.

Qualls *et al* have obtained similar results which indicate removing the larger particles can increase the level of microbial inactivation. From their work, it can be concluded that the adverse effects of UV disinfection on larger particles may occur due to the presence of more resistant coliforms in bigger size particles.

2.2.6 UV Absorbance and Scattering of Microbial Floccs

As mentioned before while UV light irradiates to the solution containing solid particles, it may be absorbed, scattered, or passed through the solid materials. Figure 2.4 represents the possible incomplete penetration of UV light into wastewater particles.

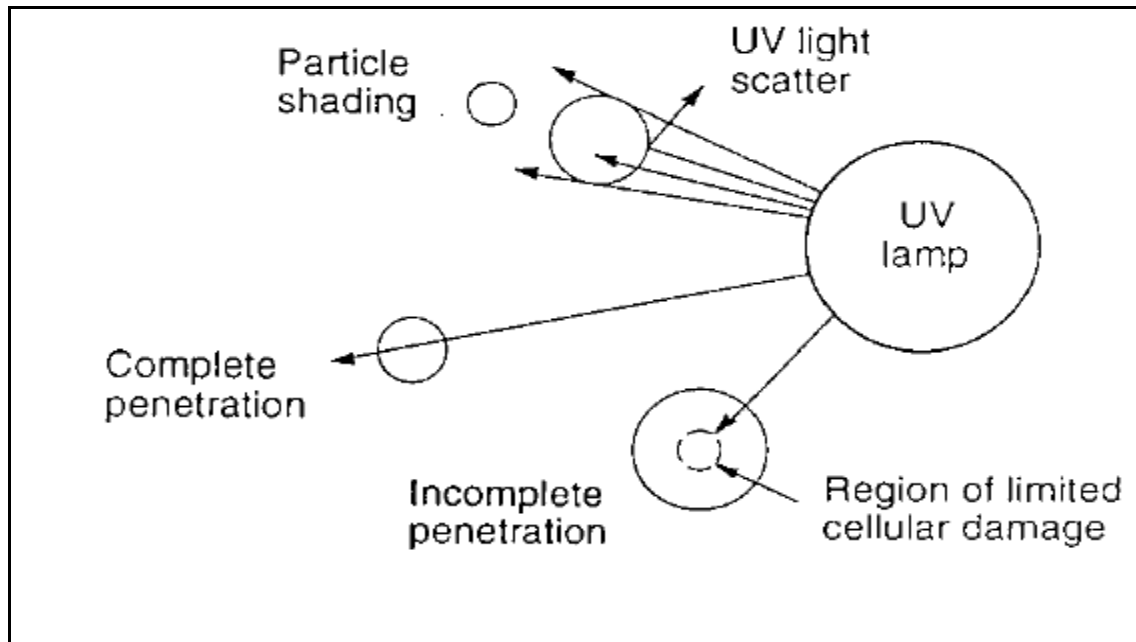


Figure 2.4. Particle Interactions that impact UV effectiveness

2.2.7 UV light penetration into wastewater particles

Loge *et al.* (1999), has reported that ultraviolet light can be highly absorbed by wastewater particles; but it can still inactivate the microorganisms by penetrating to some extent through their materials. Since wastewater particles such as activated sludge particles are highly porous it was suggested that as microbial flocs highly absorb UV light it can only penetrate through particles porosity not through the solid material.

2.2.8 Tailing Phenomenon

Tailing phenomenon usually occurs at high UV dosages due to the presence of microbial flocs, which may absorb or scatter UV light photons during their pathway through water or provide shielding for the other microorganisms and prevent UV light reaching them. In this phenomenon a quantity of the microorganisms are still active through water even after high UV light exposure time. However, tailing also occurs in chemical disinfection of wastewater where an amount of bacteria can survive due to the incomplete penetration of chemical agent into the suspended particles. Tailing phenomenon is illustrated in Figure 2.5; the figure indicates how the rate of microbial inactivation decreases at higher dosages in the tailing phenomenon.

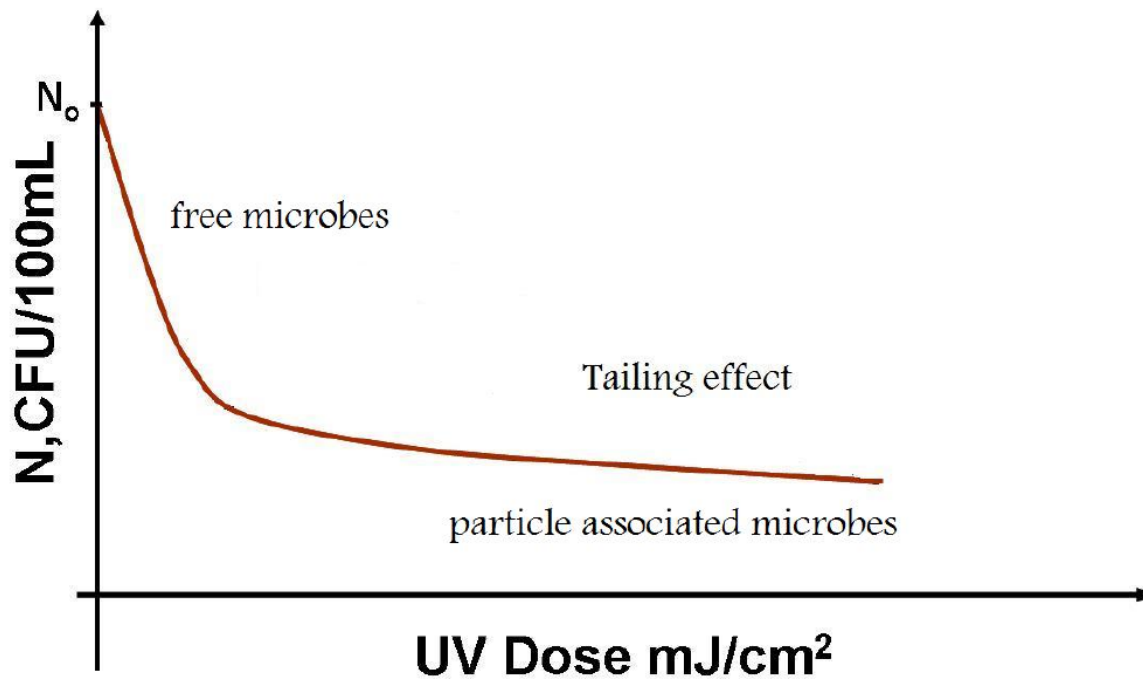


Figure 2.5. UV Dose response curve

2.3 Different types of UV lamps

Several commercially available sources of UV light are listed below :

1. Mercury vapor lamps (low, medium and high pressure)
2. Low-pressure high-output mercury vapor lamps (LPHO)
3. Electrode-less mercury vapor lamps
4. Metal halide lamps
5. Xenon lamps (pulsed UV)
6. Eximer lamps
7. UV lasers
8. Light emitting diodes (LED)

2.4 Disadvantages of UV disinfection

Microorganisms which are damaged during UV irradiation might be repaired by cell repair mechanisms. For instance, during transportation or distribution of treated water, damaged microorganisms get enough time to be regenerated and repaired. Microbial repair may increase the UV dose demand of effluent but it does not change the result.

2.5 Microbial repair in UV disinfection

Microbial repair is an enzymatic reaction that leads to DNA repairing of microorganisms. Microbial repair consists of photo reactivation and dark repair. Photo reactivation needs light for repairing the cells. To avoid this phenomenon treated water can be simply kept away from light after disinfection. Dark repair phenomenon is not as significant as the photo reactivation. Dark repair is concerned to some microorganisms repairing which does not require light for repairing but it can also happen in the presence of light. It usually occurs during water distribution through pump lines due to growth of bio film in pump lines.

2.6 Effect of temperature and pH on UV microbial response

Effect of Temperature and pH on UV microbial response extremely depends on the microorganisms types; temperature has a minimum effect on UV microbial response, in pH (6-9) microbial response is independent to the pH.

2.7 SCIENTIFIC WORK QUOTED

Behari et al (2011) made an attempt and found that Physical methods of wastewater treatment are useful in water purification and recycling. They have proposed the role of nanoparticle in combination with ultrasound. Ultrasound (US), nanoparticle (NP) or in combination (US + NP) may offer an attractive proposition in the area of water purification. Ultrasound irradiation applications is that acoustic cavitation can affect a number of mechanical, acoustic, chemical and biological changes in wastewater analysis. Their present study also defines the interaction of Reactive Oxygen Species (ROS) present as contamination in wastewater during treatment process. Owing to ultrasonication and nanoparticle treatment, ROS may generate in water samples, and disrupt the bacterial cell. Number of research has been done on water purification by using ultrasound, ultraviolet and nanoparticle. Present study established the combined effect of US + NP and suggested that it may be more effective than individual.

Kavindra Kumar Kesari et al (2011) made an attempt and found that Physical methods like ultrasound, ultraviolet and nanoparticles are very useful in wastewater purification and recycling. The ultrasound irradiation in a liquid leads to the acoustic cavitation phenomenon, which can affect a number of mechanical, acoustic, chemical and biological changes in waste analysis. In present study, ultrasonic irradiation treatment technique was used to treat the sewage sludge effluent, which was collected from Delhi and another sample of pure *E-coli* strain was processed. Samples were treated in ultrasonic bath at 35 and 130 kHz of irradiation for different time periods of 5 min, 10 min, 20 min and 30 min including control (untreated). Treated samples were tested for different parameters, viz. bacterial cell count, chemical oxygen demand (COD), degree of disintegration COD (DDCOD), scanning electron microscope (SEM), optimum density (OD) of cells and reactive oxygen species (ROS). Result shows significant disintegration in ultrasonic treated sewage sludge and *E-coli* samples as compared with untreated (control). We observed an increased level of ROS and decreased bacterial population in treated samples with 35 kHz and 130 kHz of frequency. Result suggests that the ultrasonic treatment was more effective by increasing time and frequency. Study concludes that low-frequency ultrasonic bath at 130 kHz is more effective as compared to 35 kHz.

Manoj Kumar Singh et al (2010) made an attempt and found that Sludge is a by-product of waste-water treatment and contains multitude of pollutants. In the present study, irradiation with ultrasound as treatment technique was carried out to treat complex effluent paper sludge, sugar sludge and sewage sludge samples from different industrial places. Waste water samples were treated in ultrasonic bath at 25 kHz for different time periods of 5 min, 10 min, 20 min and 30 min. Treated wastewater samples were tested for different parameters, viz. particle size reduction by particle size analyzer, chemical oxygen demand (COD), degree of disintegration COD, ammonical nitrogen and total phosphorus. Results show that the extent of disintegration by ultrasonication in wastewater samples was different in the three cases. Sewage sludge had maximum disintegration followed by paper and sugar sludge. It is concluded that low-frequency.

Dhermendra K. Tiwari et al (2008) made an attempt and found that Silver nanoparticles are known to be good antibiotic agents. In this study, silver (Ag) nanoparticles (~6 nm) were synthesized using electro-exploding wire (EEW) technique. Antibacterial action of Ag nanoparticles was studied both in liquid and solid phase using colony forming unit (CFU) detection. Time and dose-dependent study of Ag nanoparticles shows that the effectiveness of particles increases with increasing particle dose and treatment time. This effect was dose dependent and more pronounced against Gram-negative bacteria compared to Gram-positive bacteria. Transmission electron microscopy result shows particle binding with bacterial cell membrane. Membrane potential assay and cytoplasm diffusion assay show the effectiveness of Ag nanoparticle used in this study. Ultrasonication is very effective in decreasing the bacterial population in industrial wastewater samples.

David Grewell et al (2007) made an attempt and found that Municipal wastewater sludge, particularly waste activated sludge (WAS), is more difficult to digest than primary solids due to a rate limiting cell lysis step. The cell wall and the membrane of prokaryotes are composed of complex organic materials such as peptidoglycan, teichoic acids, and complex polysaccharides, which are not readily biodegradable. Physical pretreatment, particularly ultrasonics, is emerging as a popular method for WAS disintegration. The exposure of the microbial cells to ultrasound energy ruptures the cell wall and membrane and releases the

intracellular organics in the bulk solution, which enhances the overall digestibility. This review article summarizes the major findings of ultrasonic application in WAS disintegration, and elucidates the impacts of sonic treatment on both aerobic and anaerobic digestion. This review also touches on some basics of ultrasonics, different methods of quantifying ultrasonic efficacy, and some engineering aspects of ultrasonics as applied to biological sludge disintegration. The review aims to advance the understanding of ultrasound sludge disintegration and outlines the future research direction. There is general agreement that ultrasonic density is more important than sonication time for efficient sludge disintegration. Published studies showed as much as 40% improvement in solubilization of WAS following ultrasonic pretreatment. Based on kinetic models, ultrasonic disintegration was impacted in the order: sludge pH > sludge concentration > ultrasonic intensity > ultrasonic density. Both laboratory and full-scale studies showed that the integration of an ultrasonic system to the anaerobic digester improved the anaerobic digestibility significantly.

Shen Jinfeng et al (2007) made an attempt and found that the influence of ultrasound pretreatment on sludge anaerobic digestion, the ultrasound disintegration of residual sludge in water treatment of petrochemical plant was studied, and the mechanisms of ultrasound and medium were introduced. Experimental results indicate that ultrasound cavitation induces the rise of sludge temperature, which improves ultrasound disintegration on sludge. Ultrasound pretreatment can advance observably the quantity of chemical oxygen demand in sludge supernatant fluid (SCOD), which increases with ultrasound intensity and sonication time. The degree of ultrasound disintegration increases with the specific energy input. When the specific energy input is 10 000 kJ/kg of total dry solids, the degree of ultrasonic sludge disintegration reaches 40%.

Paola Foladori et al (2006) made an attempt and found that The application of sonication to wastewater or sludge contributes to the dispersion of aggregates, the solubilization of particulate matter with an increase in its biodegradability, the damage of microorganisms due to the loss of cellular membrane integrity. This research was aimed at investigating the effects of sonication at 20 kHz frequency on viability of microorganisms present in raw wastewater and activated sludge taken from a municipal waste-water treatment plant, as well as pure strains of

Escherichia coli and *E. faecalis*. Flow cytometry was applied for the identification and quantification of viable and dead bacteria free in the bulk liquid, after the fluorescent staining of cellular nucleic acids. The main results showed that: (i) cells of *E. coli* were highly sensitive to sonication, even at low specific ultrasonic energy (E_s), and disintegration of a large amount of cells was observed; (ii) on the contrary *E. faecalis* were more resistant than *E. coli*, even if high levels of E_s were applied; (iii) bacteria in raw wastewater exhibited a dynamic of viable and dead bacteria similar to *E. coli*; (iv) in activated sludge samples, low levels of E_s produced a prevalent disaggregation of flocs releasing single cells in the bulk liquid, while disruption of bacteria was induced only by very high levels of E_s .

Seungmin Na et al (2006) made an attempt and found that the dewaterability and physiochemical properties of digested sludge after treatment with ultrasonic energy for the purpose of reducing sludge. The study involved laboratory experimentation under varying test conditions of treatment time, volume of sludge and ultrasonic energy, which combined can be denoted as specific supplied energy (E_v). Results of the experiments show that particle size (dp_{50} , dp_{10} , U) of the ultrasonically treated sludge decreases due to the separation of sludge flocs. Capillary suction times (CSTs) decrease significantly, while turbidity, VDSs/VS and SCODs/TCOD increase with ultrasonic treatment. From these results, it was found that the ultrasonic treatment specified by the supplied energy (E_v) can not only improve dewaterability but also reduce the volume and mass and change the chemical properties of sludge.

Mohammad Hadi Dehghani (2005) made an attempt and found that the impact of sonication as a disinfection method for determining the effectiveness of ultrasound waves on the inactivation of *E. coli*. Ultrasound waves at a frequency of 42 kHz were used to expose aqueous suspension of *E. coli*. Ultrasound waves display a strong influence on the rate of *E. coli* disruption in water. Inactivation occurs most at the highest sonication time. This study show that sonication in 42 khz is capable to some degree for inactivation E Coli.

Fen Wang et al (2005) made an attempt and found that Ultrasonic energy can be applied as pre treatment to disintegrate sludge flocs and disrupt bacterial cells' walls, and the hydrolysis can be improved, so that the rate of sludge digestion and methane production is improved. In this paper, by adding NaHCO_3 to mask the oxidizing effect of $\bullet\text{OH}$, the mechanisms of disintegration

are investigated. In addition, kinetics models for ultrasonic sludge disintegration are established by applying multi-variable linear regression method. It has been found that hydro-mechanical shear forces predominantly responsible for the disintegration, and the contribution of oxidizing effect of $\bullet\text{OH}$ increases with the amount of the ultrasonic density and ultrasonic intensity. It has also been inferred from the kinetics model which dependent variable is SCOD+ that both sludge pH and sludge concentration significantly affect the disintegration.

C. Bougrier et al (2004) made an attempt and found that In order to enhance the efficiency of anaerobic digestion, the effects of ultrasonic pretreatment have been studied on waste-activated sludge. Solubilization of chemical oxygen demand (COD), solid and nitrogen has been proved. Flocs were broken and compounds were made soluble. In the same time, particle size decreased with specific energy applied. In terms of biodegradability, ultrasound led to an increase in biogas production. Moreover, the relationship between biogas production and sludge fractions has been examined. For specific energy input lower than 3000 kJ/kg of total solids, biogas production linked to the particulate fraction of sludge was constant, even if the solids concentration decreased. On the other hand, biogas production linked to the soluble part of sludge increased with ultrasonic power.

Torben Blume et al (2003) made an attempt and found that Ultrasound application of 20 s at low density of 30 W/l changed the particle size distribution (PSD) of the samples, the mean particle diameter dropped from 70 to 11 μm . Generally it is assumed that bioparticles bigger than 50 μm are difficult to disinfect by UV. We observed that the relevant particle size range $>50 \mu\text{m}$ in samples taken from the primary clarifier was reduced by at least three-quarters by low ultrasound doses. As expected, these changes in PSD notably effect the disinfection efficiency of UV. Whereas UV treatment of secondary clarifier effluents alone led to a reduction of fecal coliforms by 2.5 log units, pre-treatment by sonication (only 5 s at densities of 50 and 310 W/l) clearly enhanced the disinfection efficiency: reductions of CFU (colony forming unit) concentration now ranged between 3.3 and 3.7 log units. They noticed an influence of the bacteria morphology on the disinfection efficiency of the combined process (US plus UV). Gram-positive streptococci seem less vulnerable to ultrasound exposure than thinner-walled gram-negative bacteria like the entire group of coliforms. The application of an ultrasound step

might be also useful in terms of cost-effectiveness. In the lab-scale tests 30 s of UV treatment alone were required to reduce the number of fecal coliforms by 3.7 log units. When applied in combination, 5 s of ultrasonic followed by only 5 s of UV irradiation had the same result and energy consumption was only 43%.

U. Neis et al (2002) made an attempt and found that Cavitation, induced by ultrasound at low frequencies, is an effective means for the disintegration of bacterial cells. Two effects can be observed: At low ultrasound doses bacteria flocs can be declumped by mechanical shear stresses, and at increased doses ultrasound cavitation has an impact on the cell walls such that they are broken. In lab scale experiments a horn sonotrode operated at 20 kHz was run in combination with a low-pressure mercury arc lamp to treat wastewater samples taken from the effluent of a municipal treatment plant. At low ultrasound intensities a drastic change in samples' particle size distribution was observed. Consequently, subsequent UV irradiation was far more efficient as the number of large particles which impede disinfection processes was minimized by the sonication. Hence, applied UV doses could be reduced notably to obtain the same or even better disinfection effects.

C. P. CHU et al (2000) made an attempt and found that the effects of ultrasonic treatment on the physical, chemical, and biological characteristics of a waste-activated sludge. A critical ultrasonic power level exists above which, accompanied with the release of divalent cations from the sludge body, the floc structure effectively disintegrated, microbial level acceptably disinfected, and particulate organic compounds sufficiently transformed into soluble state. Both ultrasonic vibration and bulk temperature rise contribute to the treatment efficiency. Possible mechanisms of ultrasonic treatment are discussed.

CHAPTER-3

MATERIALS & METHODOLOGY

3.0 Experimental Design

The methodology adopted for this research work was categorized in seven parts:

- 1) Sample collection and preparation
- 2) Waste Water Characterization
- 3) Characterization of nanoparticle
- 4) Application of Ultrasonication on Waste Water
- 5) Application of UV on Waste Water
- 6) Application of nanoparticles on Waste Water
- 7) Combined effects of Ultrasonication, nanoparticles & UV on Waste Water.

The field work involved sample collection from a highly polluted open drain near Vasant Kunj, New Delhi. Water quality analysis was done by analyzing COD (Chemical Oxygen Demand), NH_3N (Ammonical Nitrogen analysis), Heterotrophic Plate technique for bacterial enumeration and Total Solids analysis as per APHA, 1998. Standard methods for the examination of water and waste-water: American Public Health Association, American Water Works Association, Water Environment Federation, USA. Characterization of nanoparticles involves determining the shape, size using TEM. Part 4,5,6 & 7 of this research work involved application of Ultrasonication, Nano-Ag & UV to Waste Water and to study their effects on the physical, chemical properties of the waste water.

Whole experiment has been grouped in Ultrasound, Ultrasound (US) + Ultraviolet Light (UV-C) & Nanoparticle (Nano-Ag).

3.1 SAMPLE COLLECTION & PRESERVATION

Waste Water samples were collected in polyethylene bottles from a highly polluted drain near Vasant Kunj, New Delhi. Samples for all parameters viz COD, NH₃N, Microbiological examination were collected in separate bottles. For COD and NH₃N estimation bottle was completely filled with zero head space to exclude air and was transported under ice away from light. Preservation was done with H₂SO₄ to make pH<2. For Microbiological analysis sample was collected in plastic bottles and >25 cm head space (for mixing) was kept in bottle, sample was transported under ice and was preserved with .0008% Na₂S₂O₃. The collected samples were kept in ice chest and transported to laboratory, where they were kept at 4°C until further analysis.

3.2 Wastewater Characterization

3.2.1 COD (Chemical Oxygen Demand) (APHA, 1998. Standard methods for the examination of water and waste-water: American Public Health Association, American Water Works Association, Water Environment Federation, USA).

Method of Selection for COD Estimation- Open reflux method

Chemical oxygen demand (COD) is defined as the amount of a specified oxidant that reacts with the sample under controlled conditions. The quantity of oxidant consumed is expressed in terms of its oxygen equivalence. COD often is used as a measurement of pollutants in wastewater and natural waters.

Principle:

Most types of organic matter are oxidized by a boiling mixture of chromic and sulfuric acids. A sample is refluxed in strongly acid solution with a known excess of potassium dichromate (K₂Cr₂O₇) for 2 hours. After digestion, the remaining unreduced K₂Cr₂O₇ is titrated with ferrous ammonium sulfate to determine the amount of K₂Cr₂O₇ consumed and the oxidizable matter is calculated in terms of oxygen equivalent.

Apparatus

a. Reflux apparatus, consisting of 500- or 250-mL erlenmeyer flasks with ground-glass 24/40 neck and 300-mm jacket Liebig, West, or equivalent condenser with 24/40 ground-glass joint, and a hot plate having sufficient power to produce at least 1.4 W/cm² of heating surface, or equivalent.

b. Blender.

c. Pipettes, Class A and wide-bore.

Reagents

a. Standard potassium dichromate solution, 0.04167M: 12.259 g K₂Cr₂O₇ was dissolved, primary standard grade, previously dried at 150°C for 2 h, in distilled water and diluted to 1000 ml. This reagent undergoes a six-electron reduction reaction; the equivalent concentration is $6 \times 0.04167\text{M}$ or 0.2500N.

b. Sulfuric acid reagent: Ag₂SO₄, reagent or technical grade, crystals or powder were added to conc. H₂SO₄ at the rate of 5.5 g Ag₂SO₄/kg H₂SO₄. It was allowed to stand for 1 to 2 days to dissolve and was mixed.

c. Ferroin indicator solution: 1.485 g 1,10-phenanthroline monohydrate and 695mg FeSO₄·7H₂O were dissolved in distilled water and diluted to 100 ml.

d. Standard ferrous ammonium sulfate (FAS) titrant, approximately 0.25M: 98 g of Fe(NH₄)₂(SO₄)₂·6H₂O was dissolved in distilled water and 20 ml of conc. H₂SO₄ was added, cooled, and diluted to 1000 mL. This solution was standardized daily against standard K₂Cr₂O₇ solution as follows: 25.00 ml of standard K₂Cr₂O₇ was diluted to about 100 ml. 30 ml conc. H₂SO₄ was added & cooled. Titrated with FAS titrant using 0.10 to 0.15 ml (2 to 3 drops) ferroin indicator.

Molarity of FAS solution

$$= \frac{\text{Volume } 0.04167M \text{ K}_2\text{Cr}_2\text{O}_7 \text{ solution titrated, mL}}{\text{Volume FAS used in titration, mL}} \times 0.2500$$

e. Mercuric sulfate, HgSO_4 , crystals or powder.

f. Potassium hydrogen phthalate (KHP) standard, $\text{HOOC}_6\text{H}_4\text{COOK}$: KHP was lightly crushed & then dried to constant weight at 110°C . 425 mg was dissolved in distilled water and diluted to 1000 ml. KHP has a theoretical COD₁ of 1.176 mg O_2/mg and this solution has a theoretical COD of 500 mg O_2/ml .

Procedure: Sample was blended and diluted 50 times, 1 gm HgSO_4 was added followed by several glass beads and 5 ml sulphuric acid reagent was added very slowly with mixing to dissolve HgSO_4 . While mixing sample was cooled to avoid loss of volatile materials. 25 ml of 0.04167M $\text{K}_2\text{Cr}_2\text{O}_7$ solution was added and mixed. Flask was attached to condenser and cooling water was turned on. Remaining sulphuric acid reagent (70 ml) was added through open end of condenser. Swirling and mixing was continued while adding Sulphuric acid reagent.

Open end of condenser was covered with a small beaker to prevent foreign material from entering refluxing mixture and was refluxed for 2 h. Condenser was cooled and washed down with distilled water. Reflux condenser was disconnected and mixture was diluted to about twice its volume with distilled water. Sample was allowed to Cool to room temperature and excess $\text{K}_2\text{Cr}_2\text{O}_7$ was titrated with FAS, using 0.10 to 0.15 ml (2 to 3 drops) ferroin indicator. End point of the titration was taken as the first sharp color change from blue-green to reddish brown that persisted for 1 min or longer.

Calculation

$$\text{COD as mg O}_2/\text{L} = \frac{(A - B) \times M \times 8000}{\text{mL sample}}$$

where:

A= ml FAS used for blank,

B= ml FAS used for sample,

M = Molarity of FAS, and

8000 = mill equivalent weight of oxygen \times 1000 ml/l.

Initial COD of the sample – **5456 mg/l**

3.2.2 Microbiological Examination- Heterotrophic Plate Count (APHA)

The heterotrophic plate count (HPC), formerly known as the standard plate count, is a procedure for estimating the number of live heterotrophic bacteria in water and measuring changes during water treatment and distribution or in swimming pools. Colonies may arise from pairs, chains, clusters, or single cells, all of which are included in the term “colony-forming units” (CFU). The final count also depends on interaction among the developing colonies; choose that combination of procedure and medium that produces the greatest number of colonies within the designated incubation time.

Selection of Method

Spread plate method: The spread plate method causes no heat shock and all colonies are on the agar surface where they can be distinguished readily from particles and bubbles. Colonies can be transferred quickly, and colony morphology easily can be discerned and compared to published descriptions. However, this method is limited by the small volume of sample or diluted sample that can be absorbed by the agar: 0.1 to 0.5 ml, depending on the degree to which the pre-poured plates have been dried. To use this procedure, maintain a supply of suitable pre-dried, absorbent agar plates.

Media

- a. Plate count agar (Tryptone, Glucose, Yeast agar): This high-nutrient agar, widely used in the past, gives lower counts than R2A or NWRI agar.

Tryptone	5.0 g
Yeast extract	2.5 g
Glucose	1.0 g
Agar	15.0 g
Reagent-grade water	1 L

pH was maintained at 7.0 ± 0.2 after autoclaving at 121°C for 15 min.

Incubation

For compliance monitoring purposes under U.S. EPA's Surface Water Treatment Rule provision on heterotrophic bacteria, pour plates were incubated at 35°C for 48 h.

Counting & Recording: As per APHA

$$\text{CFU/mL} = \frac{\text{colonies counted}}{\text{actual volume of sample in dish, mL}}$$

Initial Bacterial Count: 7.2×10^9 CFU/ml

3.2.3 Ammonical Nitrogen Estimation N-NH_3^+ (APHA).

Initial N-NH_4^+ : 452 mg/l

3.2.4 Total Solids (APHA, 1998. Standard methods for the examination of water and wastewater: American Public Health Association, American Water Works Association, Water Environment Federation, USA).

Initial Total Solids: 10.2 mg/l

3.3 Characterization of Nanoparticles

Silver (Ag) nanoparticles (cat. no. 730785) purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA) was used in the present investigation. The purchased nanoparticle was characterized by TEM.

3.4 Application of Ultrasonication on Waste Water

Waste water samples were blended with magnetic stirrer followed by ultrasonic treatment at three different duration of time ($t_1=15$, $t_2=30$, $t_3=45$), using ELMA, multi frequency ultrasonic bath (according to manufacture Instruction). This apparatus worked with an operating frequency of 35 kHz and a supplied power of about 250W. Batch experiments were carried out in beakers without temperature regulation (no cooling). Treated samples had a volume of 5ml.

Ultrasonic pre-treatment specifications were taken as under

Treatment time (min.): 15(t_1), 30(t_2) & 45(t_3)

US Frequency: 35 kHz

US Power: 250 W.

US Intensity: Power supplied per transducer area
(50.95 watt per cm^2)

US Density: Power supplied per sample volume
(2500 watt per lit)

3.4.1 COD Solubilization

The chemical oxygen demand (COD) was determined by oxidation of the organic compounds with $\text{K}_2\text{Cr}_2\text{O}_7$. COD was measured in sewage sample by following control, sonicated (15, 30 & 45). Each sample was centrifuged for 2 hour at 10,500 rpm and the aqueous phase

supernatant was taken for further analysis. The required reagents and methodology for analysis was followed according to APHA. The determined COD volume is used to calculate degree of sewage disintegration. Waste Water samples contain millions of microorganisms (bacteria). Ultrasonic shock waves break the microbial cell walls. The intracellular compounds (protein, enzyme, fat) are released in aqueous phase resulting in an increase of chemical oxygen demand. The assessment of cellular disruption was ascertained by measuring COD (mg/l) in the supernatant of treated samples. The maximum COD released was observed at t_3 mins.

COD solubilization (S_{COD}) was determined. S_{COD} was calculated using the difference between soluble COD (COD_s) and initial soluble COD (COD_{s0}), compared to the initial particulate COD(COD_{p0}):

$$S_{COD} = (COD_s - COD_{s0})/COD_{p0} \times 100\%$$

3.4.2 Nitrogen Composition

Cells were broken due to ultrasound. Intracellular compounds were released into the liquid phase and were made soluble. So ultrasounds led to a nitrogen release.

3.4.3 Effect of Ultrasonic Power Density

The effect of ultrasonic power density on the solubilization of the NH_3-N and the COD solubilization was investigated. The wastewater solutions containing the NH_3-N of 490 mg/lmg/L and the COD of 5456 mg/l were exposed to ultrasound at various ultrasonic power densities.

3.5 Application of Nanoparticles on Waste Water

The Ag nanoparticles were suspended in triple distilled water to conduct the time-dependent antibacterial study. Ultrasonicated and UV treated waste water samples at t_1 , t_2 & t_3 were treated with 0.5 ml of each concentration ($5\mu g/ml$, $10\mu g/ml$ & $15\mu g/ml$) of Ag nanoparticles for 3 hrs, 6 hrs, 9 hrs & 12 hrs. Before using the Ag nanoparticle, the suspension was

homogenized using an ultrasonic cleaner. Each treated bacterial culture was serially diluted till 10^6 dilution factor and 100 μ l from each culture was homogeneously spread in LB agar plates. All plates were incubated at 37°C for 24 h and the number of colonies grown on agar plate was counted.

3.6 Application of UV-C on Waste Water

Low Pressure mercury vapour Lamp by Scientech was used. Microbial response is a measure of the sensitivity of the microorganism to UV light and is unique to each microorganism. UV dose-response is determined by irradiating water samples containing the microorganism with various UV doses using a collimated beam apparatus and measuring the CFU/100 ml before and after exposure.

UV dose-response relationships can be expressed as either the proportion of microorganisms *inactivated* or the proportion of microorganisms *remaining* as a function of UV dose. Microbial inactivation has a dose-response curve with a positive slope, while microbial survival has a dose-response curve with a negative slope.

3.7 Combined Effects of Ultrasonication, UV-C and Ag Nanoparticles on Waste Water

Effects of sonication at three different times t1, t2 & t3 , UV C at three different doses & Ag nanoparticles at three different doses with different exposure time on Bacterial Count was analyzed as per APHA.

Each type of sample was divided into two groups and designated as control and sonicated.

3.7.1 Ultrasound

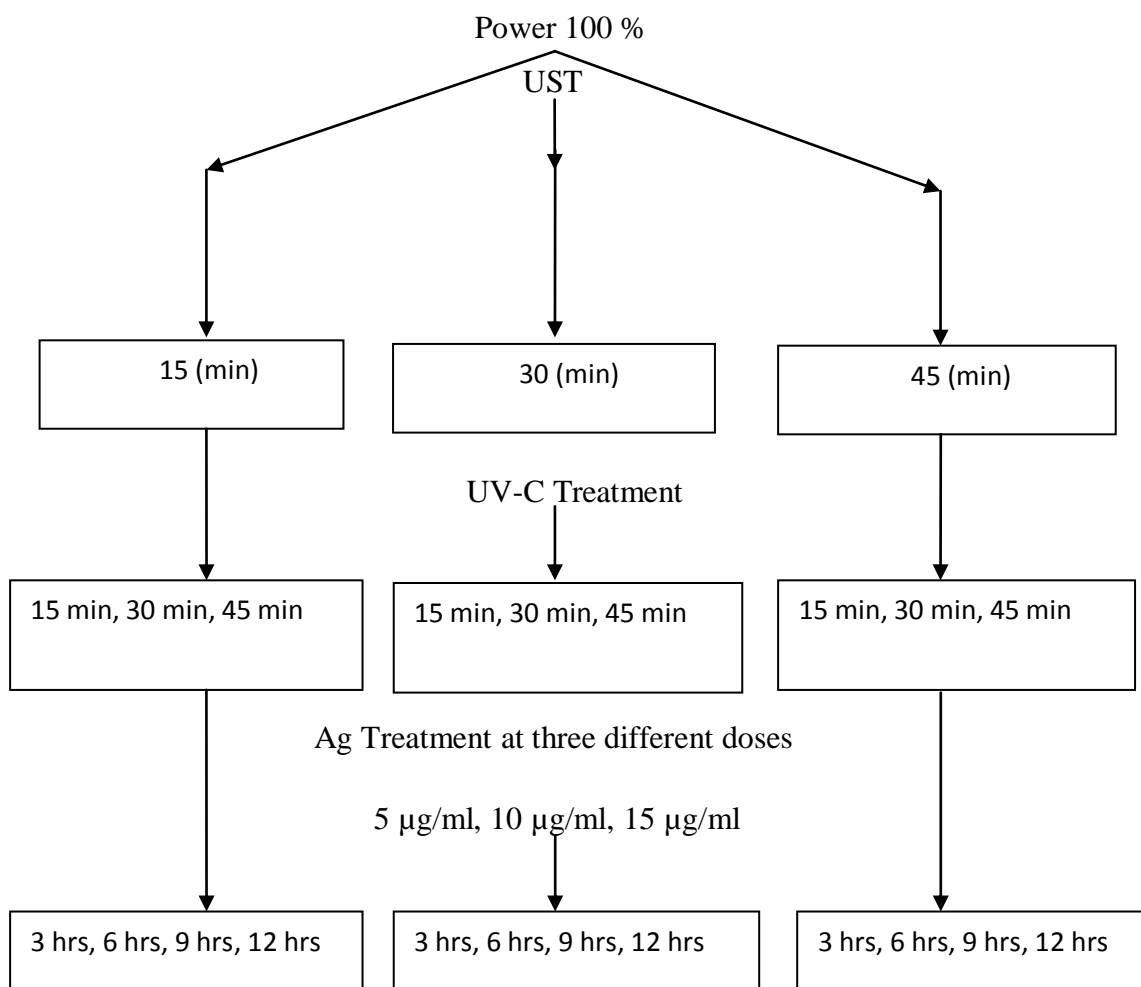
Ultrasonication at three different powers was applied to the samples as follows:

- (a) Power 50% at three different times 15, 30 & 45 mins at frequency 35kHz.
- (b) Power 80% at three different times 15, 30 & 45 mins at frequency 35 kHz.
- (c) Power 100% at three different times 15, 30 & 45 mins at frequency 35 kHz.

3.7.2 Ultrasound + Ultraviolet + Nanoparticle

Dose Optimization: Significant results were obtained at Power 100%. So, for further research work i.e (combined effect of Ultrasonication, UV & Nanoparticle) has been done using this power.

Three different doses of Ag nanoparticles have been taken viz; 5µg/ml, 10 µg/ml & 15 µg/ml and treatment time for all the doses was 3, 6, 9 & 12 hrs respectively.



UST- Ultrasonication time in minutes

UV-C- UV treatment for different times representing three different doses

Ag (hrs)- Time & Dose dependence of Ag nanoparticles

Figure 3.1. Flowchart representing the whole experiment where UST refers to Ultrasonication time, UV-C representing UV-C treatment for different times & Ag (hrs) represents three different silver dose at different treatment times as shown viz (3, 6, 9, 12 hrs).

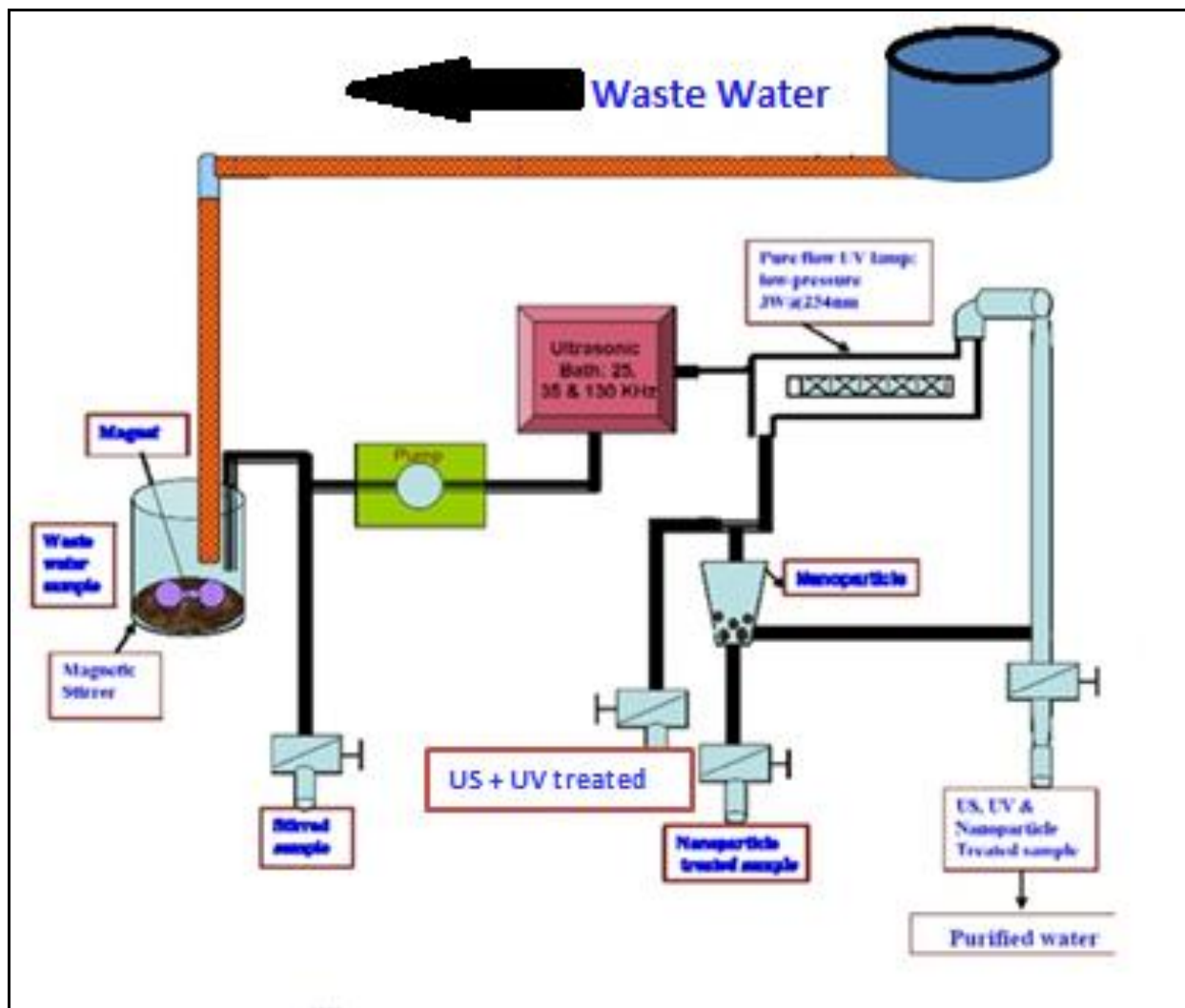


Figure 3.2. Wastewater treatment by using ultrasound, nanoparticle and Ultraviolet.

CHAPTER 4

RESULTS & DISCUSSION

4.1 Raw Sample Characteristics

Table-4.1. Raw Sample Characteristics

COD (mg/l)	5456
NH ₃ -N (mg/l)	452
Total Solids (mg/l)	10.2
CFU/100ml	7.2×10 ⁹

4.1.2 Elemental Composition of Sample

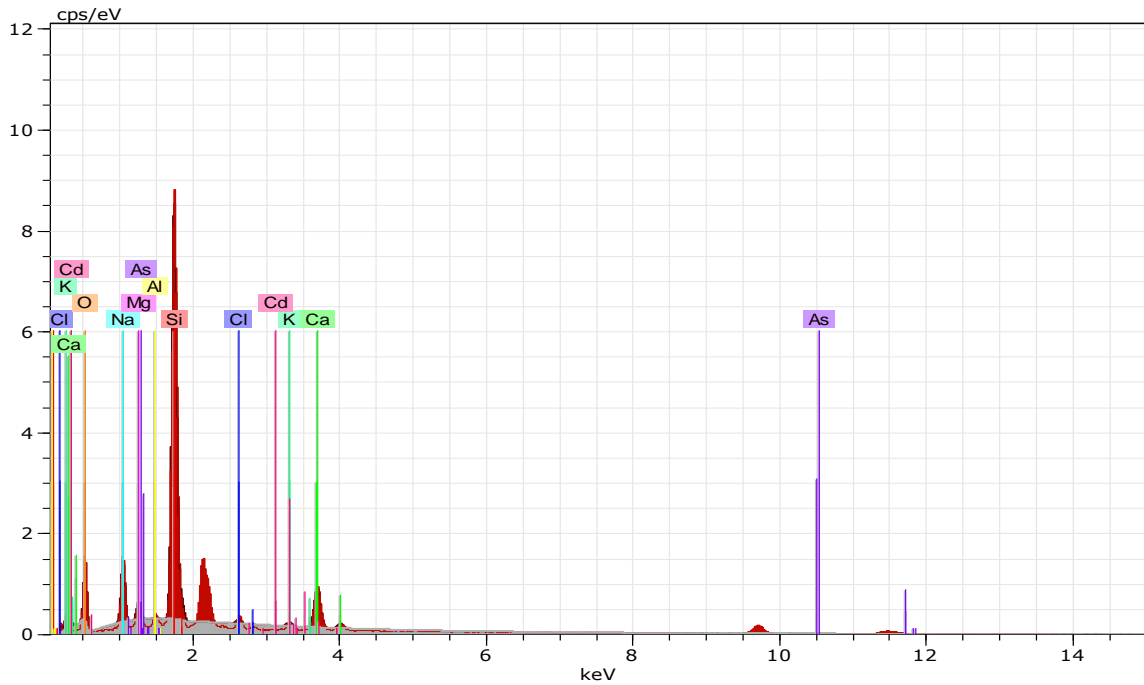


Figure 4.1 Elemental composition of raw sample estimated from SEM-EDX.

Element	Series	unn.Cnorm.C	Atom. C	Error	
		[wt.-%]	[wt.-%]	[at.-%]	[%]
Silicon	K-series	10.68	10.68	6.55	0.5
Calcium	K-series	2.38	2.38	1.02	0.1
Chlorine	K-series	0.19	0.19	0.09	0.0
Sodium	K-series	2.31	2.31	1.73	0.2
Magnesium	K-series	0.50	0.50	0.35	0.1
Aluminium	K-series	0.05	0.05	0.03	0.0
Potassium	K-series	0.22	0.22	0.10	0.0
Arsenic	L-series	0.00	0.00	0.00	0.0
Cadmium	L-series	0.00	0.00	0.00	0.0
Oxygen	K-series	83.67	83.67	90.12	0.7
Total:		100.00	100.00	100.00	

SEM EDX results show that there is no heavy metal contamination in the sample.

4.1.3 SEM Analysis of Raw Sample

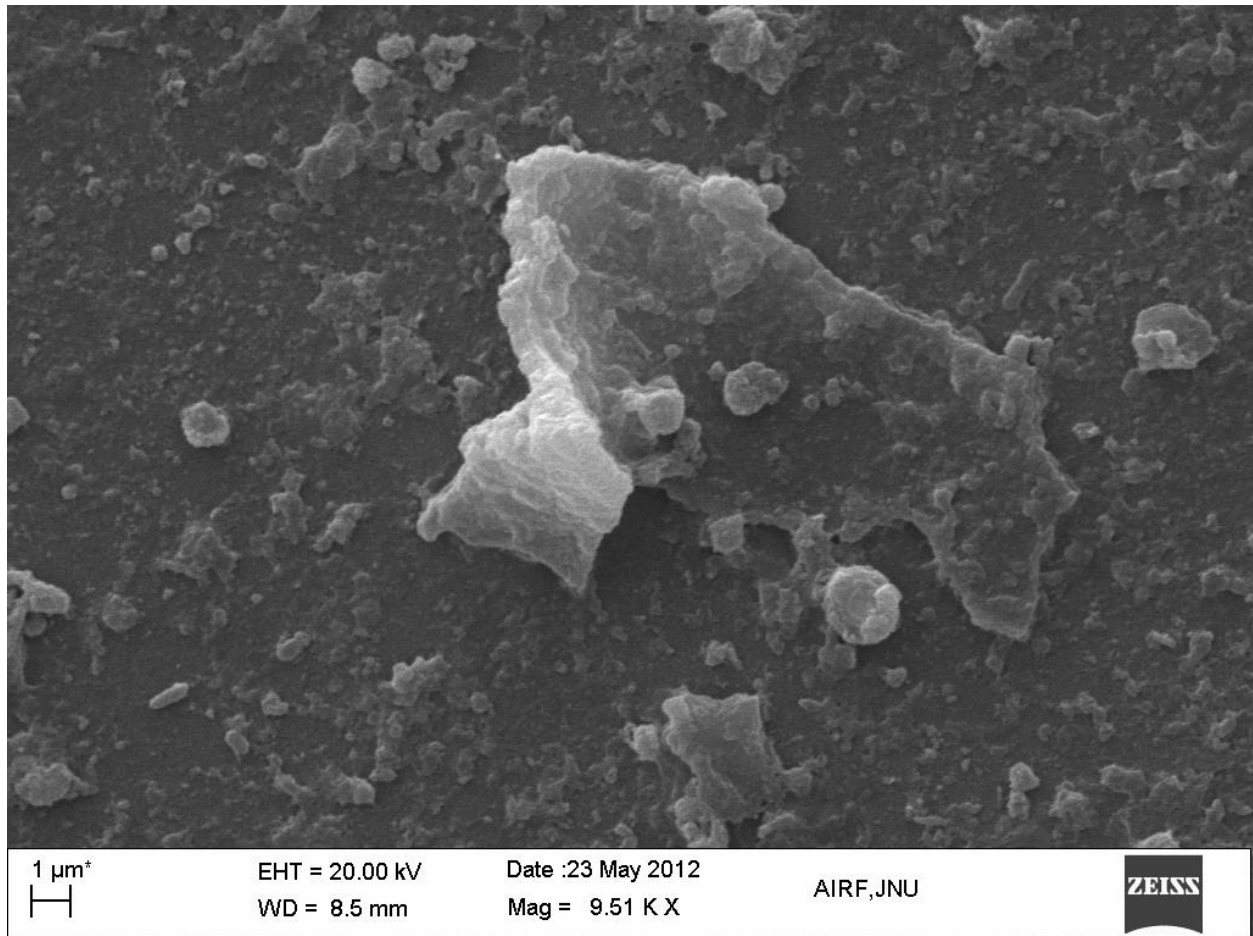


Figure 4.2: Scanning electron Microscopy of Raw Sample showing different particulate sizes and various bacteria's e.g. Rod shaped & Spherical shaped.

4.2 COD Solubilization after Ultrasonication

For each experiment, while the energy input increased, total COD was constant. During the experiments, the soluble/particulate COD repartition varied: soluble COD (CODs) increased whereas particulate COD (CODp) decreased. Cells underwent lysis and organic compounds were released into the liquid phase. CODs increased strongly for specific supplied energy between 0 and 1×10^{10} kJ/kg. For specific energy under 5.2×10^9 kJ/kg TSS, Solubilization is low (10.9%). For supplied energy E_S over 5.2×10^9 kJ/kg TSS Solubilization rose strongly; for E_S 1×10^{10} kJ/kg TSS, $S_{COD} = 29.90\%$. COD measurements are normally applied for soluble pollution at low concentration. Fig 4.3 shows the variation of COD Solubilization with supplied energy.

Table 4.2. Variation of COD Solubilization with Specific Energy

Specific Energy [kJ/kg TSS]	S_{COD} [%]
1.7×10^9	4.30%
2.8×10^9	8.60%
3.6×10^9	10.40%
5.2×10^9	10.90%
5.6×10^9	16.40%
7.0×10^9	19.60%
8.4×10^9	24.70%
1×10^{10}	29.90%

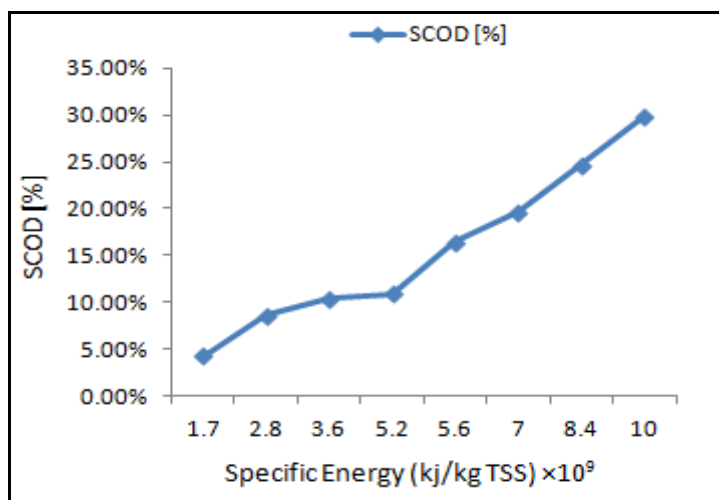


Figure 4.3. Solubilization of COD versus supplied energy

4.3 Effect of Ultrasonic Power density on NH₃-N

Cells were broken due to ultrasound. Intracellular compounds were released into the liquid phase and were made soluble. So ultrasounds led to a nitrogen release. The effect of ultrasonic power density on the removal efficiencies of the NH₃-N was investigated. The wastewater sample containing the NH₃-N of 452 mg/L was exposed to ultrasound at various ultrasonic power densities. Nitrogen is mainly in proteins or amino acids. Proteins were made soluble but were not completely degraded. It is proposed that the removal of the NH₃-N proceeds mainly via the reactions of thermal decomposition in the cavitation bubbles. Figure 4.4 shows variation of ammonical Nitrogen with supplied energy.

Table 4.3 Ammonical Nitrogen distribution as function of specific energy

Specific Energy (kj/kg TSS)	NH4 + (mg/l)
0	452
1.7×10^9	468
2.8×10^9	450
3.5×10^9	452
5.2×10^9	458
5.6×10^9	463
7×10^9	466
8.4×10^9	464
10×10^{10}	450

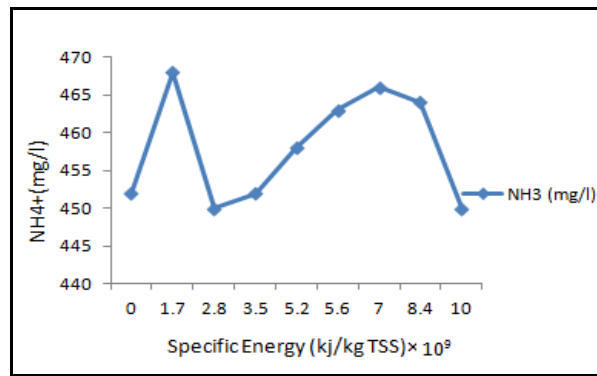


Figure 4.4 Effects of Ultrasonic Power density on the removal efficiencies of the NH₃-N .

4.4 UV Dose Response curve

A linear initial slope at low UV doses corresponding to an exponential decay in CFUs, followed by a near-plateau region at high UV doses known as the tailing region .

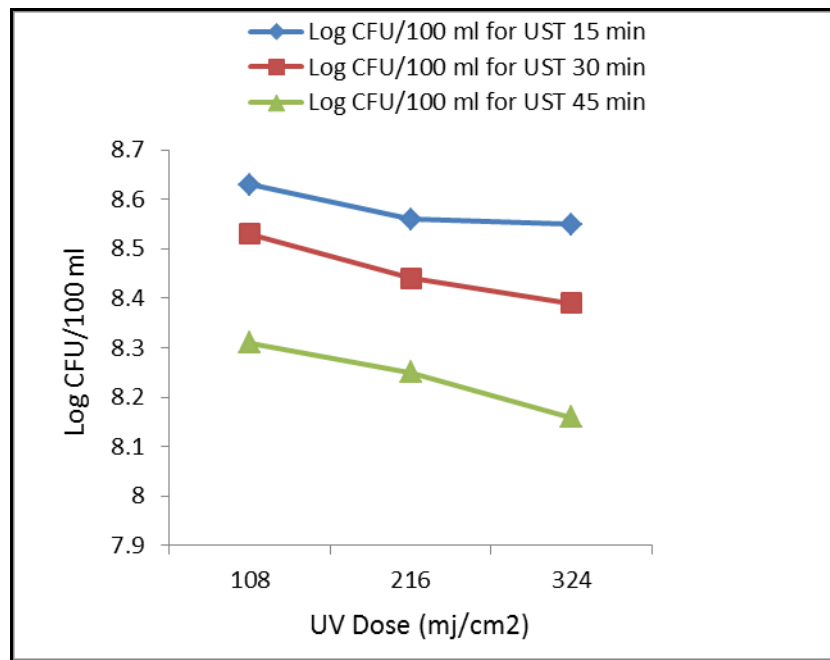


Figure 4.5 UV Dose response curve after different Ultrasonication times.

UST- Ultrasonication time (mins)

4.4 XRD & TEM results of Ag Nanoparticles employed in the study

XRD and TEM results of Ag nanoparticles used in this study are shown in Figures 4.5.2 and 4.5.1. Figure 4.5.2 shows XRD data of the Ag nanoparticles. The peaks at $2\theta = 38.14^\circ, 44.34^\circ, 64.54^\circ$ and 77.47° can be assigned to reflections from the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes respectively, of metallic silver in FCC phase. Figure 4.5.1 (a) shows a representative TEM image of a large number of Ag nanoparticles at a magnification of 30,000. Though it appears that the size of such particles is in the range 10 nm or larger, we believe that these larger particles are composed of van der Waals clusters of smaller entities. To demonstrate this we show in Figure 4.5.1(b), a high resolution image (magnification = 800,000) of two almost spherical particles joined in the middle. From geometry, it is clear that these individual particles are 6–7 nm in diameter, while the composite particle in lower resolution would appear to be in the range 20 nm. The high resolution TEM data show crystal planes and this further supports XRD analysis for the crystalline nature of Ag nanoparticles.

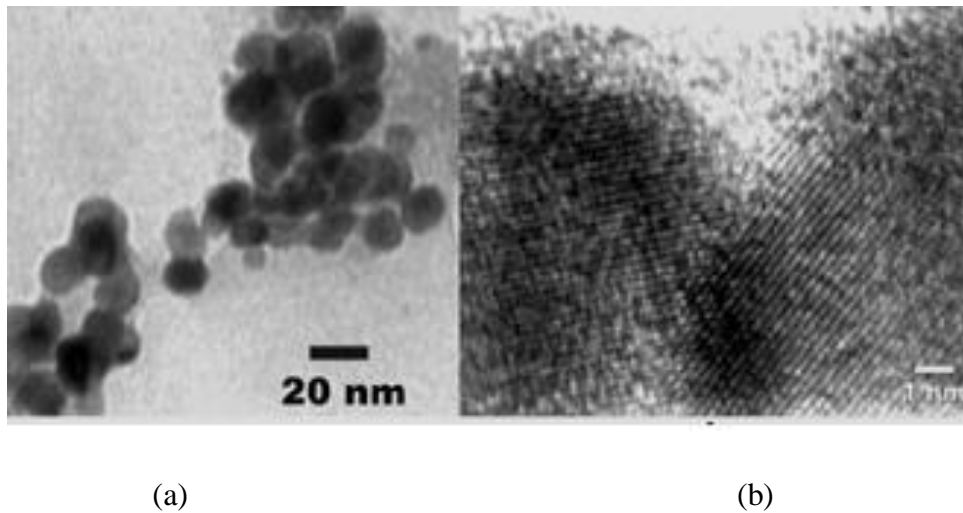


Figure 4.5.1 : a, TEM image of a large collection of nanoparticles. b, Higher magnification from a selected region showing van der Waals cluster¹⁵ of two particles, each about 6 nm across

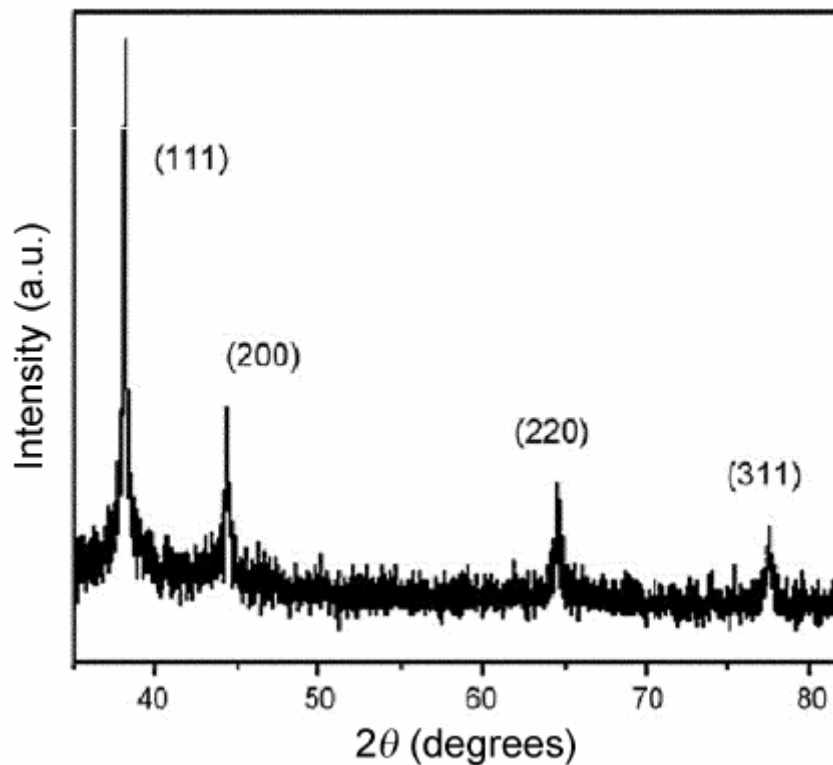


Figure 4.5.2 XRD Pattern of Ag Nanoparticles employed in the study

4.5 Combined effect of Ultrasonication, UV & Ag nanoparticles on Waste Water

It had been found that the less number of growth appeared on the Petri plate when sample was treated with Ultrasonication, along with UV light and Ag nanoparticles. A continuous decrease in the number of colonies was obtained on the agar plate as the Ultrasonication time, UV dose and nanoparticle dose + treatment time were increased. Best results were obtained at Ultrasonic treatment for 45 mins, and UV dose of 324 mj/cm^2 & Ag dose of $15 \mu\text{g/ml}$ with treatment time of 12 hrs (Figure-4.5.11). These treatment times were sufficient to inhibit 75% bacterial growth.

Around 2 log inactivation was achieved. It had been found that the less number of colonies appeared on the Petri plate treated with Ag nanoparticle, along with ultrasonic irradiation & UV-C irradiation as compared when the culture was treated with Ultrasonication alone.

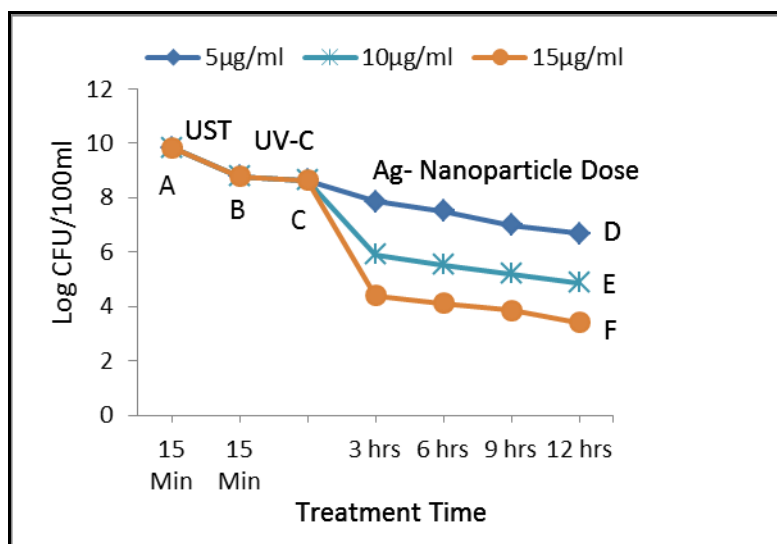


Figure 4.5.3 Growth inhibition curves of bacteria in LB medium treated with Ultrasonication for about 15 mins, UV-C treatment with dose of 108 mj/cm² (15 min treatment) & different concentrations of Ag-NPs solution.

A-B → Sample treated with Ultrasonication for 15 minutes

A-C → Ultrasonically treated sample with UV-C light treatment with dose of 108 mj/cm²

A-D → Ultrasonically+ UV treated sample with Ag Nanoparticles with dose of 5µg/ml for different treatment times viz. (3,6,9&12 hrs).

A-E → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 10µg/ml for different treatment times viz. (3,6,9&12 hrs).

A-F → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 15µg/ml for different treatment times viz. (3,6,9&12 hrs).

Ultrasonication lead to 10% kill of bacterial population further UV lead to 10.2% kill of bacterial population and subsequent Ag treatment with different doses viz. (5µg/ml, 10µg/ml & 15µg/ml) lead to more effective treatment up to 60.5% bacterial kill was seen.

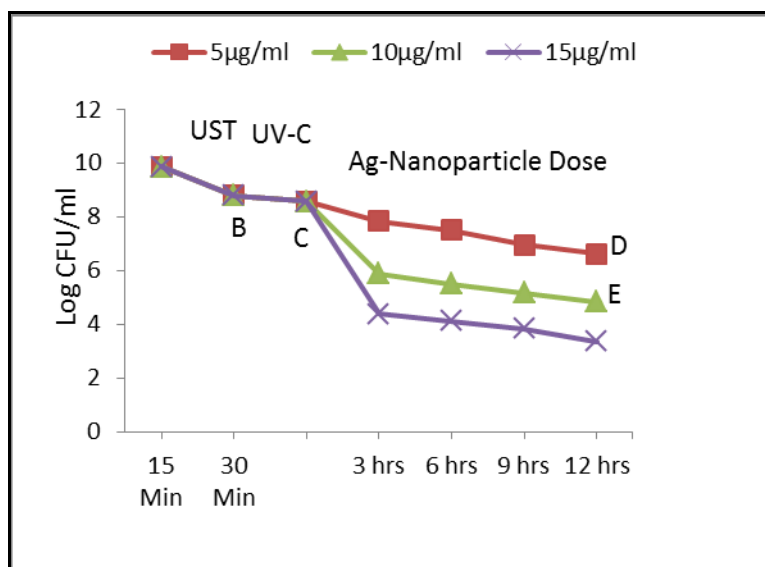


Figure 4.5.4 Growth inhibition curves of bacteria in LB medium treated with Ultrasonication for about 15 mins, UV-C treatment with dose of 216 mj/cm² viz (30 mins treatment) & different dose + treatment time of Ag-NPs.

A-B → Sample treated with Ultrasonication for 15 minutes

A-C → Ultrasonically treated sample with UV-C light treatment with dose of 216 mj/cm²

A-D → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 5µg/ml for different treatment times viz. (3,6,9&12 hrs).

A-E → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 10µg/ml for different treatment times viz. (3,6,9&12 hrs).

A-F → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 15µg/ml for different treatment times viz. (3,6,9&12 hrs).

Ultrasonication lead to 10% kill of bacterial population further UV lead to 10.4% kill of bacterial population and subsequent Ag treatment with different doses viz. (5µg/ml, 10µg/ml & 15µg/ml) lead to more effective treatment up to 60.54% bacterial kill was seen.

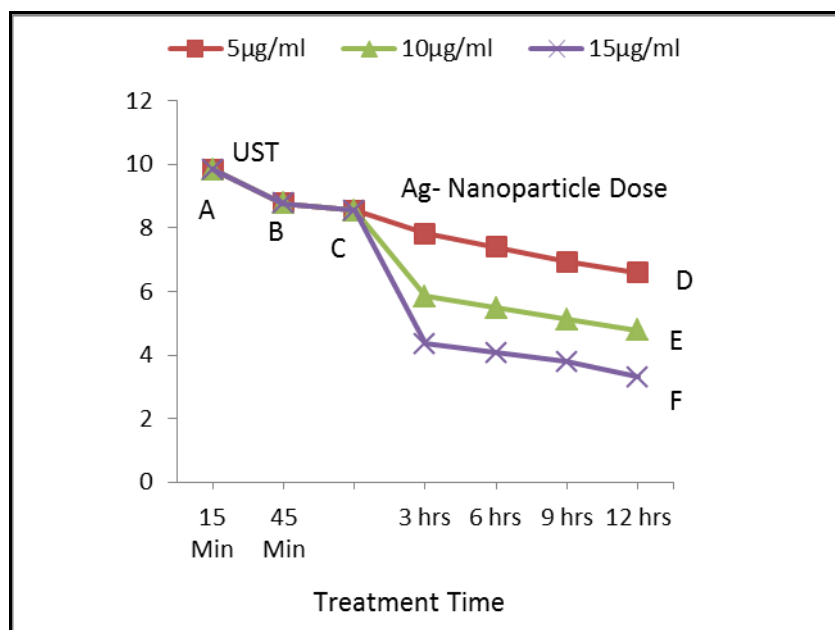


Figure 4.5.5 Growth inhibition curves of bacteria in LB medium treated with Ultrasonication for about 15 mins, UV-C treatment with dose of 324 mj/cm²viz (45 mins treatment) & different doses + treatment time of Ag-NPs.

A-B → Sample treated with Ultrasonication for 15 minutes

A-C → Ultrasonically treated sample with UV-C light treatment with dose of 324 mj/cm²

A-D → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 5µg/ml for different treatment times viz. (3,6,9&12 hrs).

A-E → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 10µg/ml for different treatment times viz. (3,6,9&12 hrs).

A-F → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 15µg/ml for different treatment times viz. (3,6,9&12 hrs).

Ultrasonication lead to 10% kill of bacterial population further UV lead to 10.4% kill of bacterial population and subsequent Ag treatment with different doses viz. (5µg/ml, 10µg/ml & 15µg/ml) lead to more effective treatment up to 60.6% bacterial kill was seen.

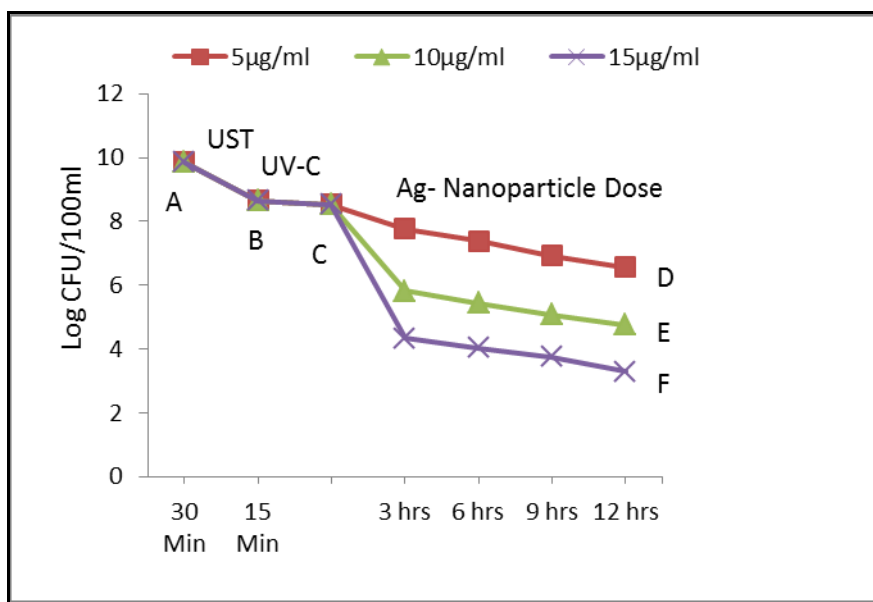


Figure 4.5.6 Growth inhibition curves of bacteria in LB medium treated with Ultrasonication for about 30 mins, UV-C treatment with dose of 108 mj/cm² viz.(15 mins) & different doses + treatment time of Ag-NPs.

A-B → Sample treated with Ultrasonication for 30 minutes.

A-C → Ultrasonically treated sample with UV-C light treatment with dose of 108 mj/cm².

A-D → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 5µg/ml for different treatment times viz. (3,6,9&12 hrs).

A-E → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 10µg/ml for different treatment times viz. (3,6,9&12 hrs).

A-F → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 15µg/ml for different treatment times viz. (3,6,9&12 hrs).

Ultrasonication lead to 10.3% kill of bacterial population further UV lead to 10.4% kill of bacterial population and subsequent Ag treatment with different doses viz. (5µg/ml, 10µg/ml & 15µg/ml) lead to more effective treatment up to 60.6% bacterial kill was seen.

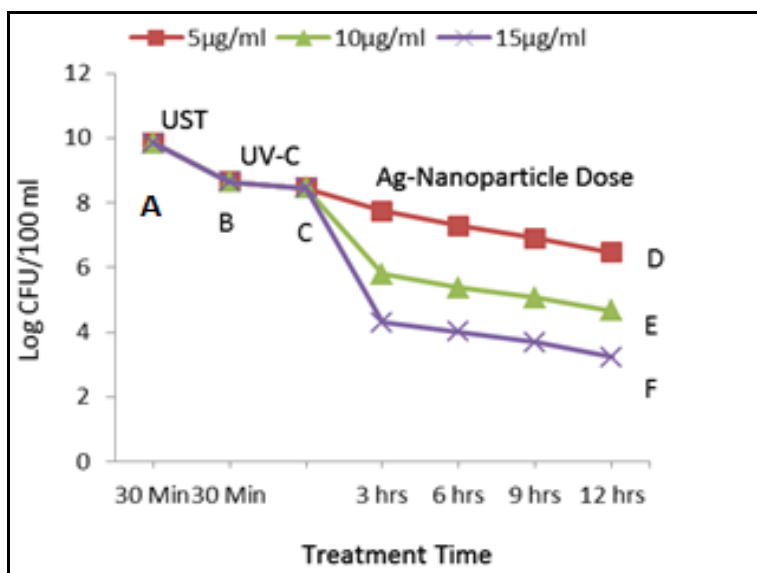


Figure 4.5.7 Growth inhibition curves of bacteria in LB medium treated with Ultrasonication for about 30 mins, UV-C treatment with dose of 216 mj/cm² viz.(30 mins treatment) & different doses + treatment time of Ag-NPs.

A-B → Sample treated with Ultrasonication for 30 minutes.

A-C → Ultrasonically treated sample with UV-C light treatment with dose of 216 mj/cm².

A-D → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 5µg/ml for different treatment times viz. (3,6,9&12 hrs).

A-E → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 10µg/ml for different treatment times viz. (3,6,9&12 hrs).

A-F → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 15µg/ml for different treatment times viz. (3,6,9&12 hrs).

Ultrasonication lead to 10% kill of bacterial population further UV lead to 10.5% kill of bacterial population and subsequent Ag treatment with different doses viz. (5µg/ml, 10µg/ml & 15µg/ml) lead to more effective treatment up to 60.7% bacterial kill was seen

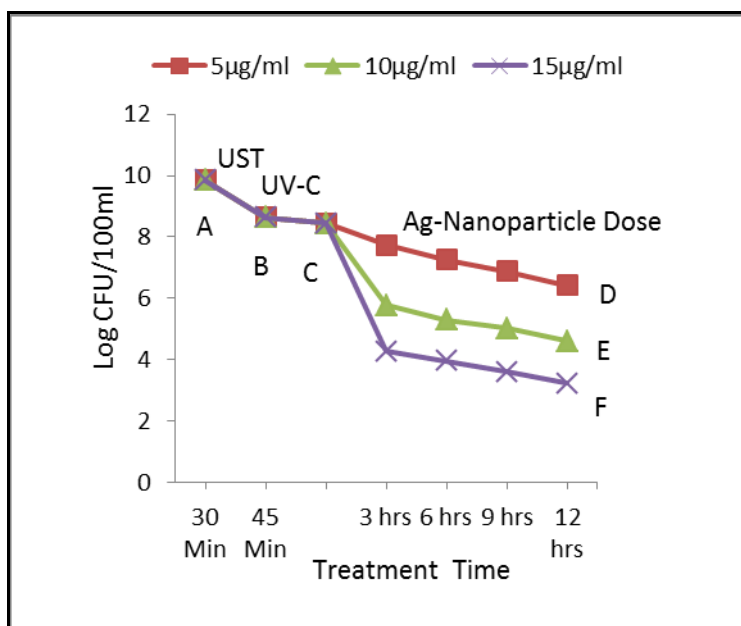


Figure 4.5.8 Growth inhibition curves of bacteria in LB medium treated with Ultrasonication for about 30 mins, UV-C treatment with dose of 324 mj/cm² viz. (45 mins treatment) & different doses + treatment time of Ag-NPs.

A-B → Sample treated with Ultrasonication for 30 minutes.

A-C → Ultrasonically treated sample with UV-C light treatment with dose of 324 mj/cm².

A-D → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 5µg/ml for different treatment times viz. (3,6,9&12 hrs).

A-E → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 10µg/ml for different treatment times viz. (3,6,9&12 hrs).

A-F → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 15µg/ml for different treatment times viz. (3,6,9&12 hrs).

Ultrasonication lead to 10.3% kill of bacterial population further UV lead to 10.6% kill of bacterial population and subsequent Ag treatment with different doses viz. (5µg/ml, 10µg/ml & 15µg/ml) lead to more effective treatment up to 60.8% bacterial kill was seen.

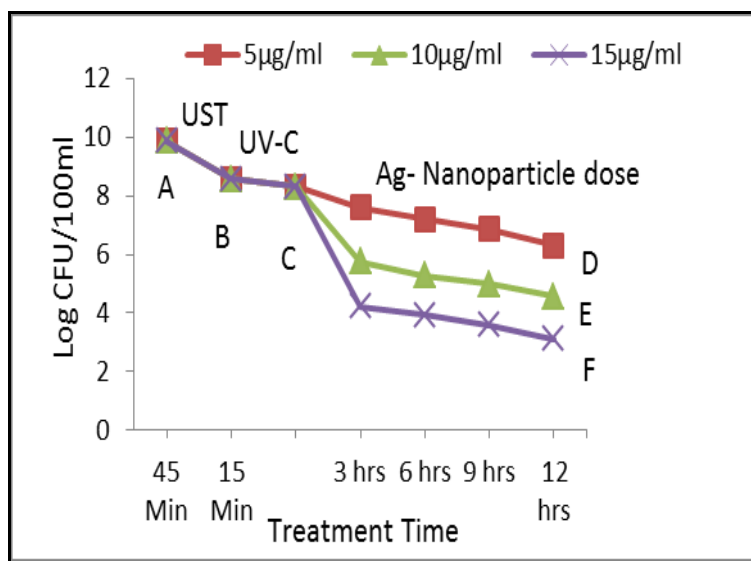


Figure 4.5.9 Growth inhibition curves of bacteria in LB medium treated with Ultrasonication for about 45 mins, UV-C treatment with dose of 104 mj/cm² viz & different dose + treatment time of Ag-NPs.

A-B → Sample treated with Ultrasonication for 45 minutes.

A-C → Ultrasonically treated sample with UV-C light treatment with dose of 104 mj/cm².

A-D → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 5µg/ml for different treatment times viz. (3,6,9&12 hrs).

A-E → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 10µg/ml for different treatment times viz. (3,6,9&12 hrs).

A-F → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 15µg/ml for different treatment times viz. (3,6,9&12 hrs).

Ultrasonication lead to 10.3% kill of bacterial population further UV lead to 10.6% kill of bacterial population and subsequent Ag treatment with different doses viz. (5µg/ml, 10µg/ml & 15µg/ml) lead to more effective treatment up to 60.8% bacterial kill was seen.

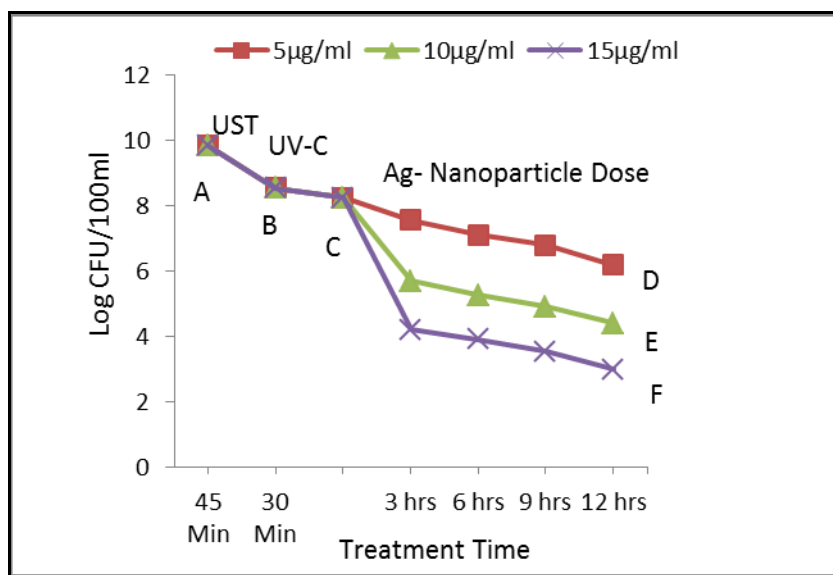


Figure- 4.5.10 Growth inhibition curves of bacteria in LB medium treated with Ultrasonication for about 45 mins, UV-C treatment with dose of 216 mj/cm² viz. (30 mins treatment) & different doses + treatment time of Ag-NPs.

A-B → Sample treated with Ultrasonication for 45 minutes.

A-C → Ultrasonically treated sample with UV-C light treatment with dose of 216mj/cm².

A-D → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 5µg/ml for different treatment times viz. (3,6,9&12 hrs).

A-E → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 10µg/ml for different treatment times viz. (3,6,9&12 hrs).

A-F → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 15µg/ml for different treatment times viz. (3,6,9&12 hrs).

Ultrasonication lead to 10.3% kill of bacterial population further UV lead to 10.7% kill of bacterial population and subsequent Ag treatment with different doses viz. (5µg/ml, 10µg/ml & 15µg/ml) lead to more effective treatment up to 60.9% bacterial kill was seen.

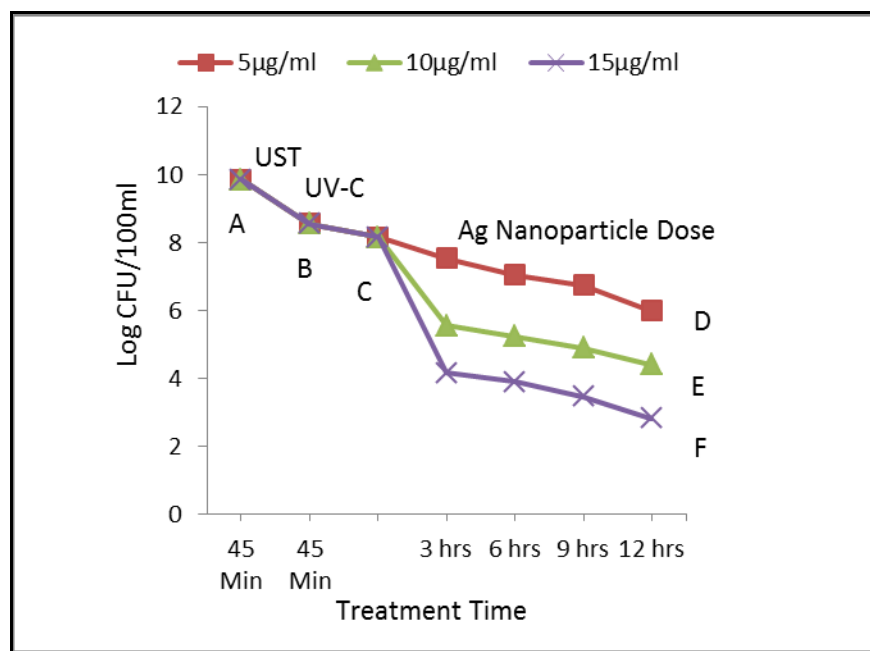


Figure- 4.5.11 Growth inhibition curves of bacteria in LB medium treated with Ultrasonication for about 45 mins, UV-C treatment with dose of 324 mj/cm² viz. (45 mins treatment) & different dose + treatment time of Ag-NPs.

A-B → Sample treated with Ultrasonication for 45 minutes.

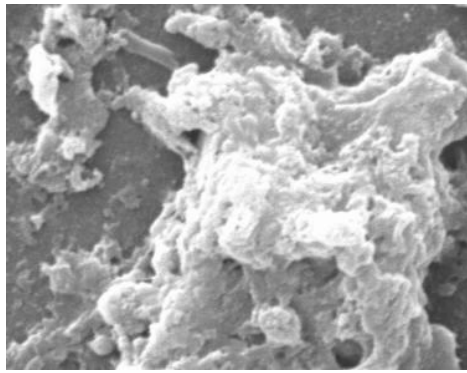
A-C → Ultrasonically treated sample with UV-C light treatment with dose of 324 mj/cm².

A-D → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 5µg/ml for different treatment times viz. (3,6.9&12 hrs).

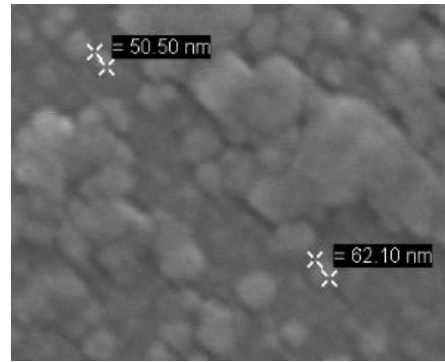
A-E → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 10µg/ml for different treatment times viz. (3,6.9&12 hrs).

A-F → Ultrasonically + UV treated sample with Ag Nanoparticles with dose of 15µg/ml for different treatment times viz. (3,6.9&12 hrs).

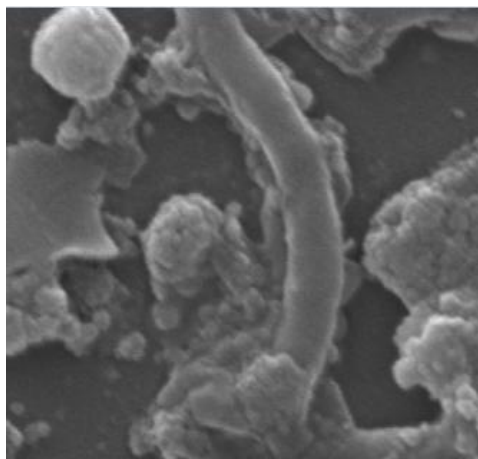
Ultrasonication lead to 10.3% kill of bacterial population further UV lead to 10.8% kill of bacterial population and subsequent Ag treatment with different doses viz. (5µg/ml, 10µg/ml & 15µg/ml) lead to more effective treatment up to 70.1% bacterial kill was seen.



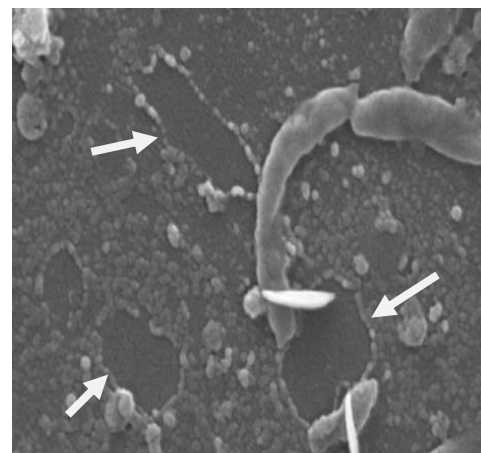
(a)



(b)



(c)



(d)

Figure 4.6 (a) SEM Image of raw sample, showing aggregated particles. (b) SEM image of sample treated with Ultrasonication for 45 minutes, UV treatment for 45 minutes & Silver dose of $5\mu\text{g/ml}$ for 12 hrs treatment, Cell fragmentation is visible. (c) SEM image of sample treated with Ultrasonication for 45 minutes, UV treatment for 45 minutes & Silver dose of $10\mu\text{g/ml}$ for 12 hrs treatment, Degradation is visible. (d) SEM image of sample treated with Ultrasonication for 45 minutes, UV treatment for 45 minutes & Silver dose of $15\mu\text{g/ml}$ for 12 hrs treatment, Complete degradation of sample is visible [Arrows pointing cell damage site].

4.6 Discussion

Ultrasound leads to the formation of dead bacterial cells. These preliminary experiments indicate that ultrasonication at the low-kHz frequency range has some efficacy in inactivating some bacterial agents that may be present in water and the process depends on sonication time, frequency and intensity of the ultrasound waves. Because ultrasound attacks the bacterial cell walls, the bacterial cells release iso-enzymes that biocatalyst hydrolytic reactions. This results in acceleration in the breakdown of organic material into smaller readily biodegradable fractions. The basic principal of ultrasound is based on the destruction of both bacterial cells membranes and difficult-to-degrade organics.

Nucleic acid is the molecule responsible for defining the metabolic functions and reproduction of all forms of life. The two most common forms of nucleic acid are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA and RNA consist of single- or double-stranded polymers comprising building blocks called nucleotides. In DNA, the nucleotides are classified as either purines (adenine and guanine) or pyrimidines (thymine and cytosine). In RNA, the purines are the same as in DNA, but the pyrimidines are uracil and cytosine. The nucleotides absorb UV light at wavelengths from 200 to 300nm. The UV absorption of DNA and RNA reflects their nucleotide composition and tends to have a peak near 260 nm and a local minimum near 230 nm. All purines and pyrimidines strongly absorb UV light, but the rate of UV-induced damage is greater with pyrimidines (Jagger 1967). Absorbed UV light induces six types of damage in the pyrimidines of nucleic acid (Setlow 1967, Snowball and Hornsey 1988, Pfeifer1997). The damage varies depending on UV dose. UV light does not destroy or damage cellular structures. Rather, UV light prevents microorganisms from reproducing. Microorganisms that cannot reproduce cannot infect and are thereby inactivated.

Synergistic effect of nanoparticles along with ultrasonic treatment shows an enhanced antibacterial effect. This stems from the fact that ultrasonic waves expand the cell wall, which allows nanoparticles to enter into the cell & UV light does not destroy or damage cellular structures. Rather, UV light prevents microorganisms from reproducing. Ag nanoparticles causes antibacterial effect by rupturing the membrane of bacterial cells at low concentration. This also allows the nanoparticles to bind to the intracellular material, thereby causing antibacterial

activity. The concentration of nanoparticles is responsible for biocidal effect along with the treatment time.

CHAPTER 5

CONCLUSIONS

To achieve the objectives of the present study, sample collected was analysed for various parameters such as COD Solubilization, Ammonical Nitrogen & Total Heterotrophic Plate Count. The present method is very effective and useful in process of waste water treatment. Ultrasound, UV & Nanoparticle shows immense potential in wastewater residual pre-treatment. Ultrasonic irradiation alone may not be suitable to treat complex wastewaters of high organic load. In this respect, the efficiency may be improved by coupling ultrasonic with other processes such as UV light & Nanoparticles. The final data were analyzed by viewing the sample with the help of Scanning Electron Microscopy. A continuous decrease in the number of colonies was obtained on the agar plate as the Ultrasonication time, UV dose and nanoparticle dose + treatment time were increased. Best results were obtained at Ultrasonic treatment for 45 mins, and UV dose of 324 mj/cm^2 & Ag dose of $15 \mu\text{g/ml}$ with treatment time of 12 hrs. The following were the major findings of the present study:

- 1) The ultrasonic process led to floc size reduction and cells lysis. It was necessary to supply enough energy in order to lyse cells. Organic substances were more available, so biodegradability was improved. Best results were obtained at $Es 1 \times 10^{10}$ kJ/kg TSS .
- 2) UV light prevents microorganisms from reproducing. Microorganisms that cannot reproduce cannot infect and are there by inactivated. As UV dose was increased from 104 mj/cm^2 to 324 mj/cm^2 bacterial inactivation was increased.
- 3) Ag nanoparticles causes antibacterial effect by rupturing the membrane of bacterial cells at low concentration. The concentration of nanoparticles is responsible for biocidal effect along with the treatment time. $15 \mu\text{g/ml}$ of Ag dose with treatment time of 12 hrs gave the best results. SEM image shows the particle binding and damage in cell wall of bacteria. Synergistic effect of nanoparticles along with ultrasonic treatment & UV Light shows an enhanced antibacterial effect. This stems from the fact that ultrasonic waves expand the cell wall & UV light stops

further reproduction of cells finally which allows nanoparticles to enter into the cell. This also allows the nanoparticles to bind to the intracellular material, thereby causing antibacterial activity.

5.1 Future researches

The futuristic approach for wastewater treatment has been defined in Figure 5.1. Experiment has to be performed by starting with primary and secondary treatment. There could be possibility that the solid waste has been removed during the process and supernatant (water content) will be followed by ultrasound, ultraviolet and nanoparticle treatment. After the treatment sample could be passed through nanofilter membrane to retrieve nanoparticle and finally recycling it.

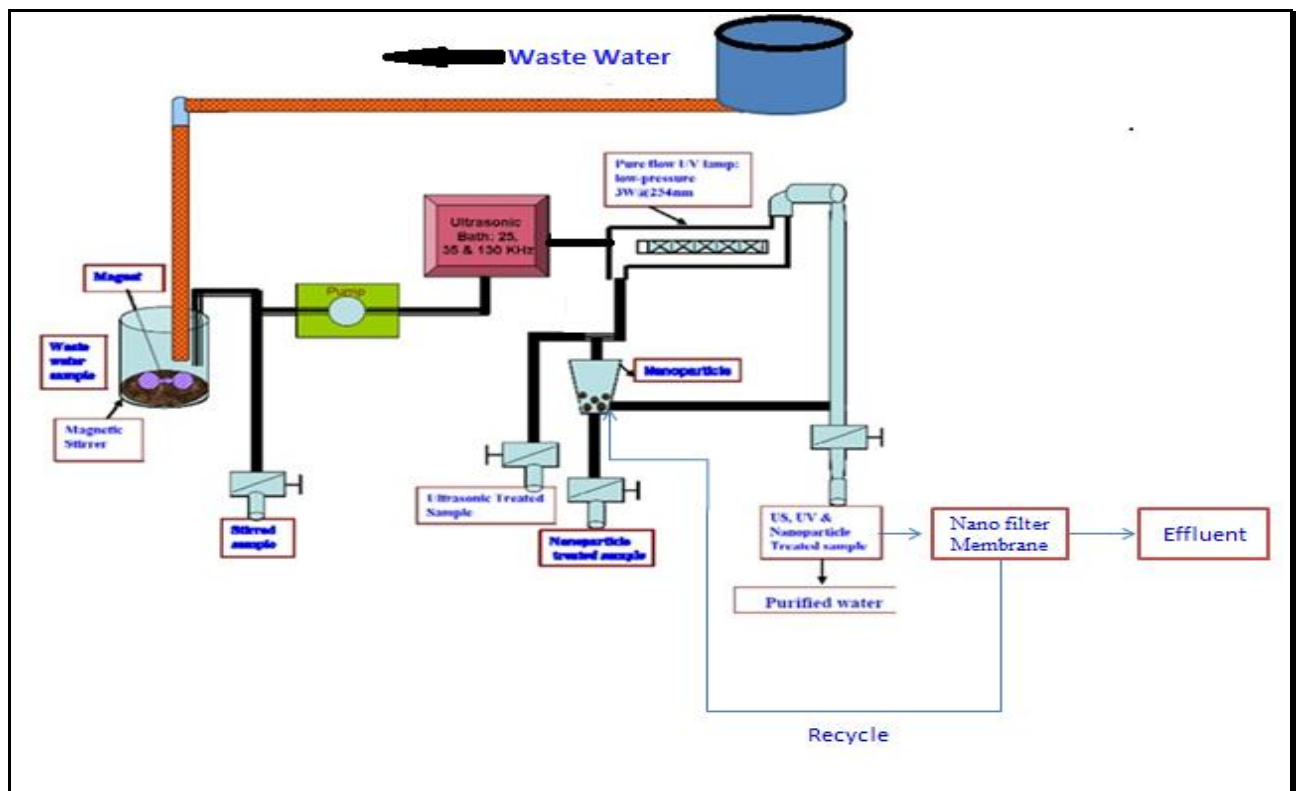


Figure 5.1 . Proposed method for wastewater treatment by using ultrasound, nanoparticle and Ultraviolet in water recycling.

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