1. Introduction

The field of nanoscience and nanotechnology is a broad and interdisciplinary area of worldwide research which develops activity that has been growing explosively in the past few years. Nanosized objects such as nanowires, nanoclusters, nanotubes, etc., open a new era of fundamental science in understanding the main relations at nanoscale. Beside these, some features of the nanosized objects like small size and a feasibility for modulation of their properties *via* self-organization into superstructures make them perhaps the most promising and attractive candidates for commercial application in future.

The fabrication of one-dimensional (1-D) nanostructures is currently one of the most attractive subjects in nanomaterial fields (Shimizu et *al*., 2009, Xia *et al*., 2003). In particular, the applicative needs for high aspect- ratio nanomaterials motivate the excellent fabrication of mesoscopic nanodevices, such as nanotubes, nanowires, nanorods, nanoribbons and 1-D nanodot arrays. Such 1-D nanostructures provide a good system to investigate electrical, thermal or mechanical properties that are produced as a direct consequence of their size, shape and reduced dimensionality. An impressive variety of methodologies have been developed to make the 1-D nanostructures, most of which may be classified into so-called soft- and hard-template syntheses (Jung and yoon, 2004). The soft-template method generates the 1-D nanostructures usually in solutions that contain functional surfactants or polymers directing the growth of certain crystal faces of the materials. The hard template method represents a straight forward route resulting in 1-D nanostructures. In this approach, the templates simply serve as scaffolds, where different 1-D materials form by replication or nanocasting.

The advantage of the hard-template method is its ability to produce complex 1-D nanostructures and facile control of the composition and dimension (Martin *et al*., 1996).

As the field of nanotechnology develops, inspiration is drawn from nature, with the evolution in biological systems of self assembling nanostructures through millions of years of molecular selection (Seeman and Belcher, 2002). The building blocks of living organisms provide valuable miniature tools, as with proteins, attractive due to the complex, reproducible structures formed with nanoscale dimensions. With the progress made in genetic engineering, along with the advances in amino acid chemistry, biomolecules have been exploited to develop novel functional materials (Katz and Willner, 2004, Wu *et al*., 2004, Zhang *et al*., 2002). The assembly and use of biological nanotubes, as an alternative to carbon nanotubes has been widely studied with diverse structures available (Bong *et al*., 2001). Examples include harnessing the natural binding properties of pili (Reguera *et al*., 2005) and virus-based magnetic and semiconducting nanowires (Mao *et al*., 2004).

1.1 Bacterial nanowires

Organisms extract electrons from many sources and the ensuing flow of these electrons through the cell's electron transport system charges a ''biological capacitor'' that can be used directly to power processes such as motility or indirectly to drive the synthesis of biologically useful energy such as ATP. This process, known as oxidative phosphorylation, requires a terminal electron acceptor to serve as the virtual ground. Prokaryotes use a variety of dissolved electron acceptors, such as oxygen, nitrate and sulfate that are freely accessible to intracellular enzymes. However, dissimilatory

metal-reducing bacteria are challenged by the poor accessibility of solid phase iron and manganese oxides that can serve as terminal electron acceptors and therefore extracellular electron transfer takes place (Chang *et al.,* 2006). **Various strategies of extracellular transfer are reported for metal-reducing bacteria (Lovley *et al*., 1996, Newman *et al.,* 2000) the most recent of which is *via* electrically conductive pilus-like appendages, called bacterial nanowires** (Reguera *et al*., 2005, Gorby *et al.,* 2006). The mechanism of transport in these biological nanostructures, however, remains unclear.

Most known electron transfer mechanisms that sustain living systems involve tunneling between sites of biological redox chains or super exchange-mediated tunneling that takes into account the structural complexity of the proteins involved (Gray *et al*., 2003, Davis *et al*., 2005). The past decade has brought about considerable interest in electron transport over longer distances in biomolecular assemblies, driven by experimental observations of transport in DNA (Dekker and Ratner, 2001). While still debated, various mechanisms have been proposed for long-range transport in DNA, including sequential multistep hopping and band-mediated conduction where the energy states are delocalized over the length scales. Szent-Gyorgyi (1941) suggested the idea of common energy bands with various densities of electronic states, similar to those available in semiconductors that represent a new paradigm in biological electron flow. Such thoughts have motivated this study of the electronic density of states in *Pseudomonas aeruginosa* nanowires. Cells of *P. aeruginosa* were cultured in shaking incubator and the nanowires' conductance was measured by cyclic voltometer (LSV and Impedance analysis)

Extracellular bacterial appendages or nanofilaments, including flagella, fimbriae, curli and pili, can influence motility, aggregation, biofilm formation, host-cell adhesion, cell signaling, DNA uptake and phage attachment. Recent work demonstrated that some bacterial nanofilaments are electrically conducting, raising the question as to whether pili and/or flagella play a role in promoting and extending extracellular electron transfer (EET) beyond the cell membrane (Gorby *et al*., 2006, Reguera *et al*., 2005, El-Naggar *et al*., 2008). The term "nanowire" describes a function of nanofilaments, i.e., transport of electrons, not a particular structure. The precise biological role of extracellular appendages may vary among microorganisms (Craig *et al*., 2004). Pili, present on the surfaces of all Gram-negative bacteria and some Gram-positive microorganisms, are typically used by bacteria in biofilm formation (Craig *et al*., 2004, Shime-Hattori *et al*., 2006, Varga *et al*., 2008, Van Houdt and Michiels, 2005) or as a conduit for DNA transfer between cells (Harrington and Rogerson, 1999, Marsh and Taylor 1999, Shu *et al*., 2008). The list of microorganisms that can produce nanowires is continuously being updated (Marsh *et al.,* 1999). Prior to concluding that a microorganism does or does not have nanowires, conducting measurements *via* scanning tunneling microscopy (STM) (Gorby *et al.,* 2006) or conducting atomic force microscopy (AFM) (Reguera *et al*., 2005) must be used to verify the conductivity of observed nanofilaments. TEM has been used for two decades to image bacteria with flagella and pili (Elliott *et al*., 1991, Bieber *et al*., 1998, Kirn *et al*., 2000, Konnov *et al*., 1999, Collins *et al*., 2005). TEM images provide consistent data indicating that pili are secreted from all sides of the bacterium and are more numerous and thinner (3–5 nm) than

flagella (8–12 nm) which are often polar, emanating from the end of a bacterium (Roine *et al*., 1997, Grossart *et al*., 2000, Jin *et al*., 2001).

In microbial world, microbes can generate energy using diverse strategies for cell growth and/or maintenance. Such energy generating processes can be simplified as a chain of electron-transfer reactions, by extracting electrons from an electron donor (organic foodstuffs) to the dumping of electrons to a terminal electron acceptor involving a series of redox reactions within the whole process. The basic principle of biological energy generation is based on oxidation and reduction processes involving electron transfer within cells and/or outside of the cells. In the case of electron transfer outside of the cells known as extracellular respiration or extracellular electron transfer, a number of mechanisms have been proposed although not many of them are completely understood. Some of the mechanisms have been experimentally proved to be in good agreement with the proposed theories, but there are definitely still knowledge gaps in each model yet to be filled and perhaps unknown mechanisms to be explored. Although most bacteria use soluble electron acceptors such as oxygen or carbon dioxide, some bacteria are capable of extracellular electron transfer in harsh environments where the only available electron acceptors are insoluble or poorly soluble, for instance, metal oxide minerals. In recent years, increasing attention has been paid to microbial metabolisms that involve electron transfer between cells and extracellular substrates. The recent discovery of electrically conductive bacterial nanowires produced by a variety of microbes suggests that electron transfer *via* nanowires may be widespread in nature. These bacterial nanowires related to energy distribution and communication and looking for their potential applications in nanobiotechnology and nanobioelectronics.

For many years, bacterial nanofilaments have been imaged using atomic force microscopy (AFM) and scanning electron microscopy (SEM) (Bieber *et al*., 1998, Chandler *et al*., 1980, Wolfgang *et al*., 2000). Their appearance in SEM images depends on growth conditions, the imaging method and the fixation protocol used prior to imaging. Using STM and SEM, (Gorby et al., 2006) the bacterial nanowires are described as thin single filaments with diameters 3–5 nm or thick bundles of nanowires with diameters of 50 to 100 nm in diameter that were straight and sometimes taut, tightly stretched between two or more cells. Additionally, in the experiments *P. aeruginosa* cells grown under aerobic conditions were shown to have few if any nanowires of any kind, leading to the conclusion that limited conditions were required for nanowire expression

A novel, previously unrecognized, extracellular electron transfer pathway was reported in 2005 by Reguera *et al*, (2005) that the dissimilarity metal reducing bacteria (DMRB) *Geobacter sulfurreducens* can produce extracellular appendages termed as microbial nanowires, which are electrically conductive pili, to aid in establishing contacts with and transferring electrons to Fe(III) oxides. A similar phenomenon was discovered by Gorby *et al*, (2006) in a variety of bacteria such as the dissimilarity metal reducing bacteria (DMRB) *Shewanella oneidensis* MR-1, the oxygenic photosynthetic *Cyanobacterium synechocystis* PCC6803 and the thermophilic, fermentative bacterium *Pelotomaculum thermopropioncium* that can also produce electrically conductive appendages, namely bacterial nanowires, when grown under specific conditions. It should be noted that the terms "microbial nanowires" and "bacterial nanowires" are used interchangeably in the literature and are both referred to electrically conductive appendages with nanowire architectures produced by microbes. In this thesis, the

isolation, separation and assessments of electrically conductive bacterial nanowires produced by *Pseudomonas aeruginosa* is discussed.

1.1.1 *Pseudomonas aeruginosa* **flagella**

The isolation of flagella from the bacteria having monotrichous flagellum, i.e., single flagellum at the end of the cell wall would be easier when compared to other types of flagellated bacteria. *P. aeruginosa* has monotrichous flagellum and is gram negative, aerobic and motile bacteria and found almost everywhere in the natural habitat ranging from surface waters to vegetation and soil. Hence, *P. aeruginosa* has been selected for this study. Flagellum is made up of flagellin protein (Fig. 1). Gram negative bacterial flagellum has 4 rings: 1. L.-rings associated with the lipopolysaccharides, 2. P-rings associated with the peptidoglycan, 3. M-ring embedded in the plasma membrane and 4. S-ring directly attached to the plasma membrane.
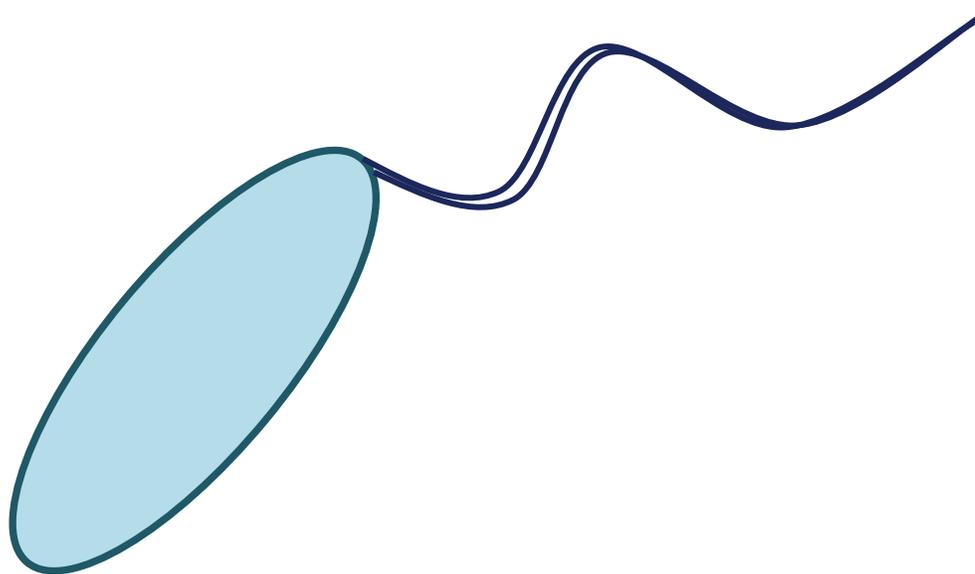

Fig. 1 Image represents the monotrichous flagellum of *P. aeruginosa.*

Bacterial flagella have the ability to form high ordered structures through self-assembly (Darnton and Berg, 2007). They are spiral and tubular nanostructures, approximately 20 nm in outer diameter, 2 nm in inner diameter and 10 μm long, which are potentially suitable as structured bio-templates (Darnton and Berg, 2007). As mechanical devices, flagella have extraordinary properties. Despite their small diameter and long length, they are extremely stiff and have an elastic modulus estimated to be in excess of 10^{10} N m^{-2} (Hoshikawa and Kamiya, 1985). Furthermore, flagella filaments are remarkably durable and remain stable at temperatures up to 60º C under relatively acidic or basic conditions (Kamiya and Asakura, 1976). Therefore, these properties allow them to be used as bio-templates either in their native or chemically and physically modified forms in a wide range of conditions.

1.2 Atomic Force Microscopy

Atomic force microscopy (AFM) belongs to the family of scanning probe microscopy that uses a laser beam deflection system where a laser is reflected from the back of an AFM cantilever with a sharp tip (probe) on the other side that is used to scan the specimen surface. The reflected beam is collected by a photodetector, which measures the beam position that contains information about the surface topography of the specimen. The typical radius of AFM tips ranges from a few to hundred of nanometers, enabling the use of AFM to observe very fine structures down to sub-nanometer scale. The working principle of AFM and its different modes of operation (contact, non-contact and tapping) as well as applications to biological sciences have been reviewed by Jalili and Laxminarayana (2004). Tapping-mode AFM allows high resolution imaging of

soft samples that are difficult to examine using contact mode, so it is widely used for imaging biological samples.

1.3 Scanning electron microscopy (SEM)

SEM is used to image bacterial cells as well as their ultrafine structures such as bacterial nanowires (Chandler *et al.,* 1980, Bieber *et al.,* 1998, Wolfgang *et al.,* 2000). SEM is one type of electron microscopy technique that images a specimen by scanning it with a beam of high-energy electrons (keV) in a raster scan pattern. The electrons interact with the surface atoms of the specimen, generating a variety of signals that contain information about the specimen's topography, composition and other properties. Conventional SEM imaging requires specimens to be electrically conductive and electrically grounded to prevent the accumulation of electrostatic charge at the surface. Non-conductive specimens can be coated with a thin layer of conductive material such as gold, platinum, osmium, carbon, *etc*., on the surface to prevent the charging effect. Since the working chamber of typical SEM is at high vacuum, specimens are normally required to be completely dry. Therefore, biological samples usually require chemical fixation and dehydration treatment to preserve and stabilize their structures.

1.4 High resolution-transmission electron microscopy (HR-TEM)

HR-TEM is used since two decades ago to image bacteria as well as their pili and flagella (Elliott *et al.,* 1991, Konnov *et al.,* 1999, Kirn *et al.,* 2000, Collins *et al.,* 2005). The high-magnification and high resolution imaging capability of TEM allows it to provide much more accurate estimation, particularly on the lateral dimensions of

nanofilaments which are usually less than 15 nm. Typically, TEM uses an electron beam with relatively high energy (~100 keV or higher) that is transmitted through the specimen where electrons can be scattered or directly pass through depending on the material property of the specimen. Therefore, the electrons emerging from the specimen carry the structural information of the specimen. Because most biological samples are nearly "transparent" to the high-energy electrons, staining the samples with heavy metals such as lead, uranium or tungsten can scatter the imaging electrons and thus enhance contrast between different structures.

TEM analysis of nanofilaments of *P. aeruginosa* was carried out in a FEI-200KeV, LaB_6 filament, Tecnai T20 G^2 TEM system. Filtered uranyl acetate (2% solution dissolved in distilled water) was used as the negative-staining reagent. Negative staining is an easy and rapid method for examining biological structures (organelles, macromolecules and viruses) at the EM level because it requires no specialized equipment. The negative stain does not react with the specimen in a "positive staining" manner. However, uranyl ions will bind to proteins and sialic acid carboxyl groups and to lipid and nucleic acid phosphate groups, providing scattering sites for incoming electrons and thus enhancing contrast of the biological structure(s). The harvested samples were applied as droplet on a TEM grid, stained with 2% uranyl acetate and dried in air after removing excess media on the grid using absorbent paper.

The conductivity of bacterial nanowires was measured using cyclic voltometry analysis – LSV and impedance techniques and their conductivity were increased after the coating of semiconductor nanoparticles over them.

1.5 Application of bacterial nanowires

The fundamental study of the electrical and electronic properties of natural organic materials, such as proteins, cells and other biomaterials, is scientifically important not only because of its significant relevance to physiology, but also because of the great potential for sensible applications in energy, environment and bioelectronics. The bacterial nanowires may represent a new category of natural, bioorganic conductive materials for potential (bio) electronic applications such as microbial fuel cells, biosensors, organic light-emitting diodes (LEDs) and organic solar cells (Leung *et al*., 2013). Raising the possibility of extracellular electron transfer through flagella, these bacteria produced nanowires might be useful in nanoelectronic applications (Davis *et al*., 2005).

Further collaborative investigations into the complete composition of nanowires, mechanisms of electron flow through the wires and interaction of nanowires between and among organisms in natural microbial communities are warranted to completely realize the implications of these structures in areas of alternative energy production, carbon sequestration, bioremediation, cell signaling and, perhaps, pathogenicity and human health (Gorby *et al*., 2006). The measurements reported here motivate further investigations into the molecular composition and physical transport mechanism of bacterial nanowires, both to understand and realize the broad implications for natural microbial systems and biotechnological applications (El-Naggar *et al.,* 2010).

1.6 Organic semiconductors

The discovery of bacterial nanowires with semiconducting characteristics opens the opportunity of natural production of bio semiconductor materials with properties comparable to commonly used synthetic organic semiconductors. To realize practical applications, the conductive nanowires should be massively produced and isolated from the bacterial cell bodies. Procedures for obtaining high amounts of isolated *Geobacter sulfurreducens* (Reguera *et al*., 2005), *Shewanella oneidensis* (Gorby *et al*., 2006) nanowires by a combination of centrifugation and vortexing are being developed (Liu *et al*., 2011). From a technological point of view, bacterial nanowires may represent a new category of natural and bioorganic conductive materials for potential bio-electronic applications such as microbial fuel cells, biosensors and organic solar cells. Recent advances, which have demonstrated longitudinal electrical transport along bacterial nanowires from the bacterial body with electrical conductivities comparable to those of moderately doped inorganic semiconductors (El-Naggar *et al*., 2010) and mechanical strength similar to polymers (Leung *et al*., 2011) have motivated further investigations into the electronic transport characteristics of these biological nanowires.

In our study, we have introduced *Pseudomonas aeruginosa* bacterial nanowires as the new bio semiconductor materials. The properties of these bacterial nanowires are comparable to already synthesized, above said *G. sulfurreducens and S. oneidensis* bacterial nanowires. This bacterial nanowire has been synthesized using simple centrifugation methods using two non ionic surfactants. The conductivity of the synthesized bacterial nanowires was measured using impedance analysis and these organic semiconductor nanowires are of low-cost and easily reproducible.

1.7 Inorganic semiconductor

When atoms assemble to form a solid and atomic states of each atom mix forming extended bands across the solid. The spacing between bands and the extent to which the electron fill the bands, determines whether a material will be a metal, insulator, or semiconductor (Fig. 2). If any energy band is full of electron, then current cannot flow and the material is an insulator. In a metal the bands are partially filled and the materials can conduct. A semiconductor resembles an insulator in that its highest occupied energy bands (valence band) is completely fill; however, the spacing between the valence band and the next empty state (conduction band) is small enough for some percentage of electron to be thermally excited to the conduction band. Therefore, the conductivity through a semiconductor can be turned by adjusting temperature and thus density of electron in the conduction band.
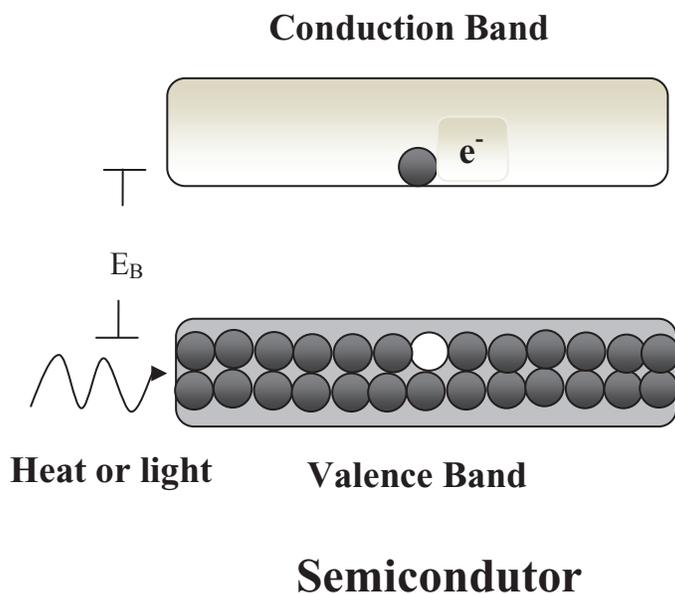
Conduction Band

e^-

E_B

Heat or light   **Valence Band**

Semicondutor

Fig. 2 Schematic diagram of Energy band for semiconductors **(Source: Porter, 2002)**

Aggregations of atoms or molecules that have at least one of their dimensions in the nano size scale (1 nm and 100 nm) indicate properties that are fundamentally different from the properties of the individual atoms or bulk matter. In recent years, there has been an increasing interest in the synthesis of nanosized crystalline metal oxides because of their large surface areas, unusual adsorptive properties, surface defects and fast diffusivities (Wu *et al*., 2002). Metal oxide semiconducting nanostructures are one of the most versatile classes of semiconducting materials due to their diverse properties and functionalities. Metal oxide nanostructures exhibit unique properties that can be used in a variety of applications for the fabrication of novel and efficient nanodevices. Therefore, rapid research developments have been made in the field of metal oxide nanostructures in terms of their growth and applications. The study of metal oxides has attracted a great deal of interest due to the importance of their size- and shape-dependent properties in electronic applications. Hence, investigations have attempted to control the morphology of metal oxide nanomaterials.

Researches in the field of fine grain mixed oxide systems have gained immense importance because of their potential applications in many areas of technology (Ozaki, 1989). Studies on the effect of temperature and frequency on the dielectric behaviour offer valuable information about conduction phenomena in nanostructured material (Mo *et al*., 1993, Nisha *et al*., 2012). The dielectric properties of oxides depend considerably on the size and shape of the particles. The micro electronics industry has been emphasizing on reduction in size of the device elements such as transistors, resistors and capacitors. However, there are practical difficulties to these achievements including the lack of ultra fine precursors to manufacture these components, poor dissipation of the

tremendous heat generated due to faster speeds and poor reliability: thus, there is a need to obtain ultra pure materials of nanometer size which have high energy density and better thermal conductivity.

Current research on nanoparticles can be classified into two main categories. The first involves manipulation and exploration of single nanoparticles in devices, for example, single electron transistors (SET) (Klein *et al*., 1997, Feldheim and Keating, 1998). The second category focuses on the application of nanoparticle assemblies to produce a new generation of devices, such as optoelectronics and photonics devices (Maier *et al*., 2001). Moreover, the nanoparticles can also be divided into two classes based on the surface capping ligands: first class composed of metallic nanoparticles with electronically active organic ligands on the surface nanoparticles in such systems act as a conducting scaffold. The second class is composed of semiconductor nanoparticles that are stabilized with long alkyl chain (which are electronically insulating) ligands and are used for construction of super lattices and glassy solids. For the study, we have chosen four different semiconductor nanoparticles based on the order of conductivity.

1. Copper oxide nanoparticles (CuO)

2. Nickel oxide nanoparticles (NiO)

3. Zinc oxide nanoparticles (ZnO)

1.7.1 Copper oxide nanoparticles (CuO)

Among different semiconducting metal oxides, copper oxide (CuO) has been studied as a unique and attractive mono-oxide material for both fundamental investigations and practical applications. CuO semiconductors exhibit a versatile range of

applications such as fabrication of electrical, optical and photovoltaic devices; selective gas sensing devices; heterogeneous catalysts; magnetic storage media; field-emission devices (e.g., field-emission gun); solar cells; and Li-ion electrode materials. Among various metal nanoparticles, copper oxide (CuO) nanoparticles have attracted considerable attention because CuO is one of the most important in modern technologies and is readily available (Pacheo *et al*., 2010). Considerable interest has been focused on copper nanoparticles due to their optical, catalytic, mechanical and electrical properties (He, 2007).

Synthesis of CuO nanostructures has been actively researched for many decades because CuO is an important industrial semiconductor due to its novel physical and chemical properties. Among all semiconductor used in modern electronic circuits, CuO is the most common one because of its excellent electrical conductivity and low cost. Logically, CuO is expected to be an essential component of nanodevices developed in future (Petit *et al*., 1998, Nasirian, 2012).

As for the special case of inorganic nanoparticles, so far, numerous methods have been developed, including (among others) solid-state reactions, sol-gel method, hydrothermal techniques and precipitation. The first three methods, although used widely, suffer from some major shortcomings. Solid-state reactions are generally associated with poor composition and morphology control. Sol-gel methods allow excellent control of composition and morphology, but its process is costly. Additional calcination and milling steps, which are required in the precipitation method, make it less desirable than the single step synthesis processed (Dawson, 1988, Outokesh *et al*., 2011). So, we have to choose a synthesis method for CuO nanoparticles in this study.

1.7.2 Nickel oxide nanoparticles (NiO)

Nickel oxide (NiO) has attracted significant notice for applications, such as catalysts (El-Molla *et al*., 2004), electrochromic film (Purushothaman and Muralidharan, 2009), gas sensors (Du *et al*., 2010), fuel cell (Li *et al*., 2004), anode of organic light-emitting diodes (Borchers *et al*., 1999), magnetic materials (Gandhi *et al*., 2011) and thermoelectric materials (Shin *et al.,* 2001), owing to its p-type conductivity, wide band gap ranging from 3.6 eV to 4.0 eV, excellent chemical stability and electrical and optical properties (Xia *et al*., 2012). On the other hand, at room temperature, stoichiometric NiO is an insulator with a resistivity of 10^{13} Ω cm. Therefore, enhancing the electrical conductivity of NiO nanoparticles is an important issue for improving the applicability of NiO nanoparticles (Guo *et al*., 2013).

Several approaches have been used to prepare NiO thin films, such as pulsed laser deposition (Yu *et al*., 2004), sputtering (Subramanian *et al*., 2008), e-beam evaporation (Hakim *et al*., 2009), electrochemical method (Chen *et al*., 2011) and sol–gel method (Raut *et al*., 2011, Guo *et al*., 2012). Among these, the sol–gel method is the most cost-effective for producing large-area films and provides excellent control of the composition and homogeneity. On the other hand, it is still challenging to develop simple, fast and versatile methods for the synthesis of highly conducting NiO nanoparticles. Sol–gel synthesized NiO nanoparticles involve many precursor solutions and sophisticated processes (Banerjee *et al*., 2003).

There are few reports on the superficial synthesis of NiO nanoparticles using a sol– gel method. The effects of the structural and electrical conductivity properties of NiO nanoparticles were examined.

1.7.3 Zinc oxide nanoparticles (ZnO)

Semiconductor ZnO with its known properties is a promising material for the wide range of advanced technological applications. The high exciton binding energy, high chemical stability and low growth temperature makes it an excellent candidate for room temperature electrical conductivity application (Cao *et al.,* 2000, Yang *et al.,* 2002). The crystal morphology and size is believed to have significant effect on the chemical/physical properties, therefore, these days much emphasis is given on the synthesis of nanostructure. ZnO nanomaterials have novel electronic, structural and thermal properties which are of high scientific interests in basic and applied fields. ZnO is a wide band gap semiconductor with an energy gap of 3.37 eV at room temperature. It has been used considerably for its catalytic, electrical, optoelectronic and photochemical properties (Suchea *et al*., 2006, Siva Kumar *et al*., 2013). ZnO nanoparticles shows different physical and chemical properties depending upon the morphology of nanostructures, not only various synthesis methods but also the physical and chemical properties of synthesized zinc oxide are to be investigated in terms of its morphology.

Many methods have been described in the literature for the production of ZnO nanostructures such as laser ablation (Scarisoreanu *et al*., 2005), hydrothermal methods (Ni *et al*., 2005), electrochemical depositions (Chang *et al.,* 2002), sol–gel method (Ristiac *et al*., 2005), chemical vapor deposition (Wu *et al*., 2002), thermal decomposition (Wang *et al*., 2009) and combustion method (Lamas *et al*., 1998). Recently, ZnO nanoparticles were prepared by ultrasound (Khorsand *et al*., 2013), microwave-assisted combust ion method (Kooti and sedish, 2013), two-step mechano

chemical-thermal synthesis (Rajesh *et al*., 2012), anodization (Shetty and Nanda, 2012), co-precipitation (Singh *et al*., 2013) and electrophoretic deposition (Vazquez *et al*., 2013). Synthesized zinc oxide nanoparticles with different morphologies by controlling different parameters of the precipitation process such as solution concentration, pH and washing medium (Rodrigues-Paez *et al*., 2001). In this study, ZnO nanostructures were synthesized using a simple precipitation method. Zinc sulfate heptahydrate and sodium hydroxide were used as precursors to formulate ZnO nanostructures.

1.8 Electrochemical measurements

The electrical measurements of bacterial nanowires fall on the discipline electrochemistry. This has been conducted using Cyclic Voltammetry (CV), Linear-Sweep Voltammetry (LSV) and Electro impedance spectroscopy (EIS) analysis.

1.8.1 Cyclic voltammetry (CV)

CV has become an important and widely used electroanalytical technique in many areas of chemistry. It is rarely used for quantitative determinations, but it is widely used for the study of redox processes, for understanding reaction intermediates and for obtaining stability of reaction products.

This technique is based on varying the applied potential at a working electrode in both forward and reverse directions while monitoring the current. For example, the initial scan could be in the negative direction to the switching potential. At that point the scan would be reversed and run in the positive direction. Depending on the analysis, one full

cycle, a partial cycle, or a series of cycles can be performed. The important parameters in a cyclic voltagram are the peak potentials (E_{pc}, E_{pa}) and peak currents (i_{pc}, i_{pa}) of the cathodic and anodic peaks, respectively.

This study is focusing on a scholarly basic exposition of cyclic voltammetry (CV) for the study of electroactive bacterial nanowires and semiconductor metal coated bacterial nanowires. The principle of cyclic voltammetry, once denoted as the spectroscopy of the electrochemist (Heinze, 1984) was being explained. Here, we focused on the mass-transfer conditions at the bacterial nanowires/electrode interface and on the principle information to be derived from its study. We focused on anodic bacterial nanowires, that is, biofilms that use the electrode as a terminal acceptor, because the mechanisms of biocathodes are only partially understood (Rosenbaum *et al.,* 2011).

1.8.2 Linear-sweep voltammetry (LSV)

LSV involves an increase in the imposed potential linearly at a constant scanning rate from an initial potential to a defined upper potential limit. LSV is of two types: hydrodynamic voltammetry and polarography. Linear-sweep voltagrams under slow sweep conditions (a few mV/s) with the solution moving past the electrode generally have the shape of a sigmoidal curve called a voltammetric wave. The constant current beyond the steep rise is called the limiting current I_l, because it is limited by the rate at which the reactant can be brought to the electrode surface by mass transport processes. The potential at which the current is equal to one half the limiting current is called the half wave potential and is given the symbol $E_{1/2}$. The half wave potential is closely related to the standard potential for the half reaction but is usually not identical to that

constant. Half wave potentials are useful for identification of the components of a solution. To obtain reproducible limiting currents rapidly, it is necessary that either the solution or the microelectrode be incontinuous and reproducible motion or a dropping mercury electrode be used. LSV in which the solution is stirred or the electrode is rotated is called hydrodynamic voltammetry. Voltammetry with the dropping mercury electrode is called polarography.

In this type of LSV, the potential is changed slowly enough and mass transfer is rapid enough that a steady state is reached at the electrode surface. Hence, the mass transport rate of analyte A to the electrode just balances its reduction rate at the electrode. Likewise, the mass transport of product P away from the electrode is just equal to its production rate at the electrode surface. There is another type of LSV in which fast scan rates are used with unstirred solutions. In this type of voltammetry, a peak shaped current-time signal is obtained because of depletion of the analyte in the solution near the electrode. Cyclic voltammetry is an example in which forward and reverse linear scans are applied. With cyclic voltammetry, products formed on the forward scan can be detected on the reverse scan if they have not moved away from the electrode or have not been altered by a chemical reaction.

1.9 Electrochemical impedance spectroscopy (EIS)

EIS is a well-established powerful technique for investigating many electrical properties of materials and their interfaces. This technique has been used for studying corrosion processes, interfacial reaction mechanismsand dynamics of mobile charge in bulk or interfacial regions of any material. The flow rate of charged particles and current

depends on the Ohmic resistance of the electrodes and the electrolyte. Large number of parameters can be deduced from EIS data; the material properties such as conductivity, dielectric constant, relaxation frequency and the interfacial properties such as adsorption-reaction rate constants, interface capacitance and diffusion coefficient. From the theoretical point of view, the impedance and the capacitance are quantities that are interesting to measure (Wagner *et al*., 1998, Manohar *et al*., 2008).

A useful tool to study the electrochemical behavior of electrode materials is electrochemical impedance spectroscopy (EIS). In this experiment, a small sinusoidal voltage stimulus of fixed frequency is applied to an electrochemical cell and its response in terms of current is measured. The ac behavior of an electrochemical system can be investigated by sweeping the frequency over several orders of magnitude (generally from a few mHz to several MHz). In the classical three electrode con figuration, the voltage is applied and measured between the working and the reference electrode while the counter electrode collects the induced current. The output impedance is the sum of the impedance due to the working electrode (WE), the interface between the WE and the electrolyte and the ohmic drop between the WE and the reference electrode (RE). In the case of lithium ion battery components, this approach has been widely used and detailed models exist to take into account the different physical, chemical and electrochemical contributions to the total electrode impedance of the cell or of one single element (positive electrode, negative electrode, or electrolyte) under investigation. The bioelectrical impedance analysis technique is a two-compartment model based on the principle that an electrical current, flows more rapidly through tissues with higher water and electrolyte content, than through biological sample which are less hydrated. Due to the greater electrolyte content

of free fat mass, it offers less resistance to electric current compared to biological samples.

It is based on the electrical principle that the volume of the conductor can be determined by measuring its length and impedance, such that $V = L / R^2$ whereby the V = volume, R = resistance and L = conductor length. By sending an electrical current through the samples, electrons flow through tissues that contain water and electrolytes. In practice, a small constant current, typically 800 μA at a fixed frequency, usually 50 kHz, is passed between electrodes and the voltage drop between the electrodes provides a measure of impedance.
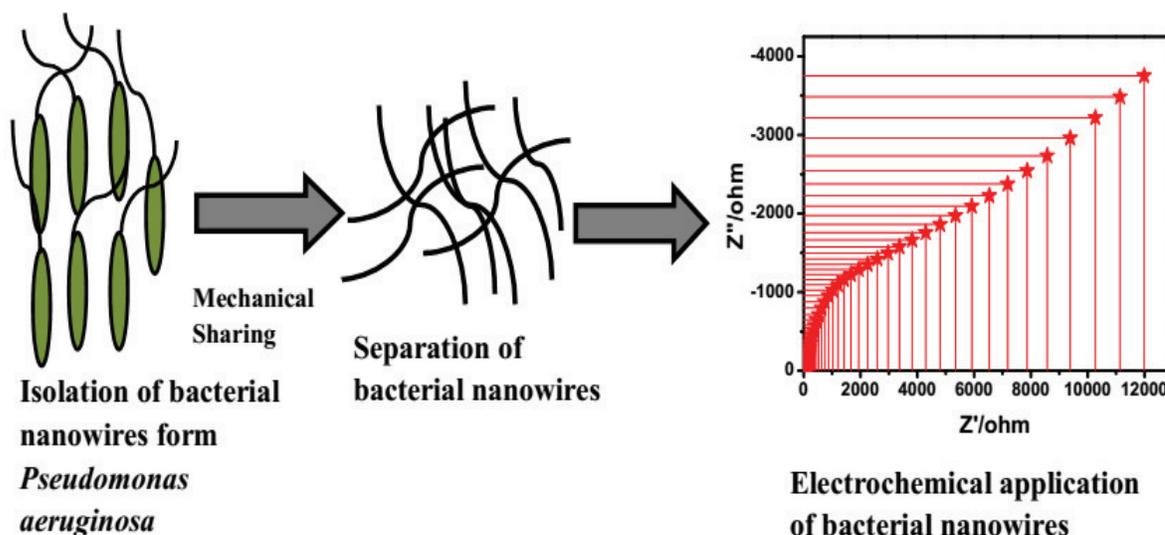

Fig. 3 Schematic representation of isolation and separation of bacterial nanowires for the electrochemical applications

To date, several biological assays have demonstrated results consistent with electron transport along bacterial nanowires including measurements of improved electricity generation in microbial fuel cells and enhanced microbial reduction of solid phase iron oxides (Reguera *et al*., 2005, Gorby *et al*., 2006, El-Naggar *et al*., 2010). However, our direct knowledge of nanowire conductivity has been limited to local measurements of transport only across the thickness of the nanowires (Reguera *et al*., 2005, El-Naggar *et al*., 2006, 2010). Thus far, there has been no evidence presented to verify electron transport along the length of bacterial nanowires, which can extend many microns, well beyond a typical cell's length. Here, we report the electron transport measurements along bacterial nanowires derived from electron-acceptor limited cultures of the *P. aeruginosa*.

In this work, we used impedance studies for electrochemical conductive measurements. Primarily, the impedance components of the bacterial nanowires are measured. To improve the electrochemical conductive properties of the bacterial nanowires, the inorganic semiconductor metal oxide nanoparticles coated onto the bacterial nanowires surface is designed and demonstrated, as schematically illustrated in Fig. 3 and 4. The significant improved electrochemical conductive properties of the bacterial nanowires were attained by the dispersion of the small size of the metal oxide nanoparticles of CuO, NiO and ZnO. The motivation of the thesis is to improve further interest in studying the electronic/electrochemical behavior of the bacterial nanowires and the molecular structures of conductive nanowires from other gram positive and gram negative microbes.
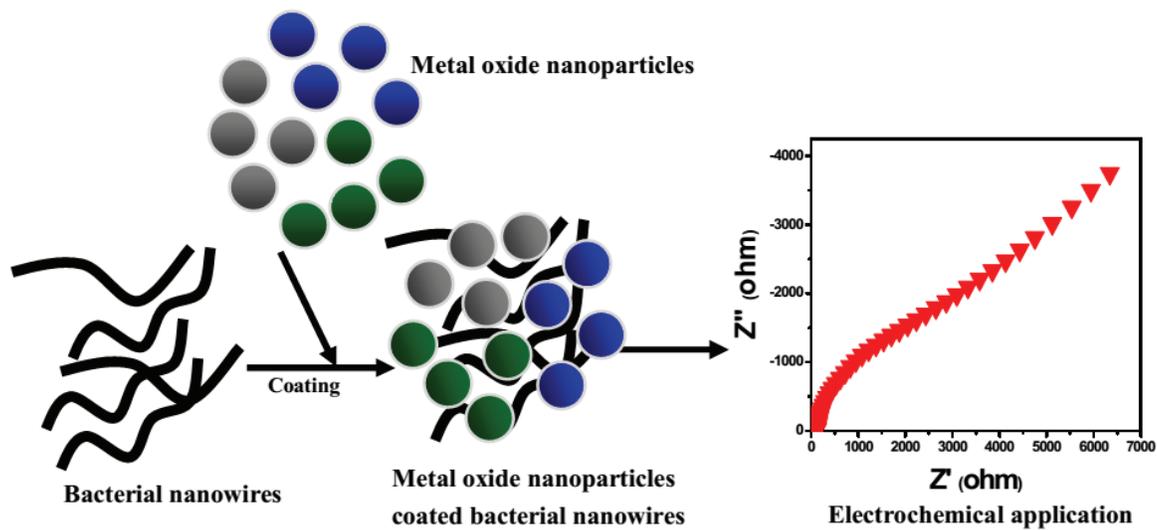
Fig. 4 Schematic representation of metal oxide nanoparticles (CuO, NiO and ZnO) coated bacterial nanowires for the electrochemical applications.