

Discussion

5. Discussion

5.1 Isolation of bacterial nanowires

The isolation and separation of bacterial nanowires and measuring its conductivity is the first of its kind to study in aerobic bacteria *P. aeruginosa*. Similar study had been carried out in anaerobic bacteria like *Shewanella oneidensis*, *Geobacter sulfurreducens* (Gorby *et al.*, 2006, El-Naggar *et al.*, 2010, Ray *et al.*, 2010, Malvankar and Lovley, 2012). In the present study, it has been reported that *P. aeruginosa* bacterial nanowires carry out extracellular electron transfer (EET) to insoluble electron acceptors such as metal oxides of anodes in microbial fuel cells. Similar studies were conducted elsewhere on *Shewanella spp.* and *Geobacter spp.* and it has been proved the electron flow from the inner to outer surface of cytochromes through EET or electron transfer promoted by membrane contact with an insoluble electron acceptor (Schuetz *et al.*, 2009, Reardon *et al.*, 2010). Isolation of mediators can also be used by these bacterial cells to do indirect EET to insoluble electron acceptors (Baron *et al.*, 2009, Coursolle *et al.*, 2010).

Isolation of nanowires connected with bacteria reportedly depends on growth conditions. Gorby *et al.*, (2006) reported that the SEM images of *Shewanella* MR-1 with attached nanowires were obtained as a result of chemostat cultures grown under O₂-limited conditions (electron acceptor limitation). Ray *et al.*, (2010) investigated that the extracellular nanofilaments in *Shewanella oneidensis* MR-1 can be accomplished with the nanofilaments still attached to the cellular bodies.

The effective separation of nanowires from the bacteria depends on the methods used to prepare the culture for imaging, as shown in Fig. 5 and 6. In a related work, no

nanowires were observed in chemostat cultures when oxygen concentrations exceeded 2% of air diffusion (Gorby *et al.*, 2006). Reguera *et al.*, (2005) confirmed that pili production in *Geobacter sulfurreducens* was growth regulated and pili were produced when the organisms were grown on Fe (III) oxide or fumarate at suboptimal temperatures below strictly anaerobic conditions. Of the two phases of *P. aeruginosa* culture used in the present study, log phase was found to be optimum for the isolation of the bacterial nanowires. The possible reason for this would be the secretion of extracellular bacterial nanowires will be efficient during the log phase (growing stage) of bacterial cells.

5.2 Separation of bacterial nanowires

Separation of flagella by mechanical shearing of *P. aeruginosa* cells with SDS resulted in flagellar bacterial nanowires of various lengths, few broken flagella and contaminations. The irregular lengths of bacterial nanowires resulted from sonicator shearing method with SDS suggested that bacterial nanowires of *P. aeruginosa* are quite delicate. In addition, these methods are highly reproducible and result in better yield of bacterial nanowires. Of the two methods followed to separate the bacterial nanowires, the sonication was found to be effective when compared to magnetic stirring process. The sonicator method used vibration to break the cell wall and to cut the flagella from the cell body. But magnetic stirrer method gave abnormal gravitational force to the bacterial cells to break them down and is displayed in Fig. 6. Hence, this method broke the bacterial cells and hence bacterial nanowires are in small pieces and difficult to observe.

Kalmokoff *et al.*, (1988) reported that obtaining flagella of various lengths was not unexpected from the sheared cell preparation but it was surprising for the nonionic

surfactant (Triton X-100) preparation as this was gentle method for recovering flagella. DePamphillis and Adler, (1971) investigated that the use of nonionic surfactant is common in usual bacterial nanowires separation techniques for eubacteria and does not appear to affect bacterial nanowires morphology. In this study, the nonionic surfactant used was SDS and the resulting image shows flagella of irregular lengths and this indicated that using SDS also reveal the similar result as Triton X-100 and is an effective method to separate bacterial nanowires (Eichfeld *et al.*, 2007).

The roughness of nanowires is important as the rough surface provides more space for the attachment of the nanoparticles/matrices and hence plays role in the efficient surface binding with other matrices. This makes a great impact in making nanowire based application devices. The roughness of stationary phase bacterial nanowires was higher than the roughness of log phase bacterial nanowires. This might be due to the size of the nanowires. Comparatively, size of the log phase bacterial nanowires was smaller than the size of the stationary phase bacterial nanowires. In addition, the time interval between the log phase and stationary phase was longer which may be the reason to develop roughness in the nanowires.

Eichfeld *et al.*, (2007) studied the bacterial nanowires of *Shewanella spp.* and compared them with lightly doped semiconductors such as silicon nanowires which are similar to polymers and this shows that *Shewanella spp.* bacterial nanowires might be used as potential candidates in the field of bio-nanoelectronics (DePamphillis and Adler, 1971). The bacterial nanowires can be investigated for the transport carrier types under nanowire transistor configurations to optimize the conductivity of individual nanowires. In the field of nanoelectronics, nanosensors and microbial fuel cells, bio-organic

nanomaterials and semiconducting bacterial nanowires will potentially be useful which in turn need further investigations.

5.3 Electrochemical studies on bacterial nanowires

5.3.1 CV studies

CV is probably the most widely used electroanalytical tool in the present investigation for examining the extracellular electron transfer (EET) process in conductive bacterial nanowires (Millo and Biochem, 2012, Harnisch and Rabaey, 2012, Khan *et al.*, 2013). CV was performed for the highly sensitive detection of EET. In a previous work done by Fricke *et al.*, 2008, Marsili *et al.*, 2008, CV was used to measure the electrocatalytic activity of bacterial nanowires, but the application of CV to bacterial nanowires requires the limiting potential range to prevent harmful oxidation or reduction conditions with the selection of informative scan rates. Marsili *et al.*, (2008) reported CV measurement at low scan rates revealed stable catalytic features of bacterial nanowires, for the CV measurements, a scan rate (20 mVs^{-1}) in $20\pm 2^\circ\text{C}$ was chosen. Under this condition, the rate of electron transfer from the bacterial nanowires to the electrode was enhanced with stable response. For examining the rate limiting steps in current generation on the bacterial nanowires, CV technique can also be used and understand the EET process in the bacterial nanowires (Katuri *et al.*, 2010, Babauta *et al.*, 2012, Khan *et al.*, 2013). Fig. 9b shows CV of the bacterial nanowires recorded to understand the electrochemical properties of the bacterial nanowires system, suggesting that the anodic and cathodic current increases in amount to the development of bacterial nanowires. No redox peaks were observed in blank, as shown in Fig. 9a. In case of bacterial nanowires electrode exhibited redox peaks, confirming that the

redox peaks were exclusively attributed to the bacterial nanowire film on the electrode surface. The reduction current appears to be high for the bacterial nanowire, because of an increase in nanowire density which enhances the number of electrons generated by oxidizing species at the surface, which in turn affects the electrochemical conductance and capacitance of the electrode.

5.3.2 LSV studies

In addition to the CV measurement, the linear polarization curves are obtained from the linear scan voltogram (LSV) in the anodic cycle to endorse the electrochemical behaviors of the bacterial nanowires thin film on the electrode surface. The anodic peak was obtained about 0.35 V for the bacterial nanowires, as shown in Fig. 10b. The almost similar result was found that anodic peak around 0.47 V corresponds to the oxidation of polyaniline present in the matrix electrode (Chauhan *et al.*, 2013). The capacitance of the bacterial nanowire coated nanowire electrode was higher than that of blank electrode, suggesting the bacterial nanowire electrode possessed a higher electrochemical activity. Therefore, growth nutrients play a crucial role in the growth of large bacterial nanowires. Fig. 10b shows the observed onset potential values and current density values at 0.19 V for the bacterial nanowires. It can be seen that the lowest more positive onset potential was observed for blank samples. It is discussed in the above that the growth of bacterial nanowires powerfully depends on medium composition and surfactant stabilization for the bacterial nanowires separation (Reguera *et al.*, 2005, Gorby *et al.*, 2006, El-Naggar *et al.*, 2010). The maximum current density was observed about 25 μA for the bacterial nanowires samples, as compared to blank samples. The maximum current density bacterial nanowires samples showed that the

bacterial nanowire electrode possessed a good electrochemical property. The bacterial nanowires thin film electrodes were stable and can be able to be reused for several times. Moreover, the repeatability of bacterial nanowires modified electrode was also examined by using the same electrode, suggesting that the bacterial nanowire system exhibited an excellent reproducibility.

5.3.3 EIS studies

EIS is generally used to examine biofilm growth, biofilm conductivity, and electron transfer mechanisms (Malvankar *et al.*, 2010, Dominguez-Benetton *et al.*, 2010). EIS is the most common alternating current method applied for the characterization of aqueous biointerfaces (Khan *et al.*, 2013). In most EIS methods, a small sinusoidal potential perturbation is applied to the sample. The frequency of this perturbation is changed in the range between a few millihertz and 100 Hz. The resulting sinusoidal current is analyzed using fast Fourier transform techniques to calculate the impedance (Z) of the interface in the frequency domain to estimate the solution resistance, polarization resistance, double layer capacitance and diffusion at the surfaces covered by protein monolayers, charge transfer time constants, and mechanisms of EET (Malvankar *et al.*, 2012, Dominguez-Benetton *et al.*, 2012, Khan *et al.*, 2013). Applications of the EIS in microbial systems, in which the sample is studied at the open circuit potential, are numerous. Therefore, EIS is performed typically at the open circuit potentials, which reveal the electron transfer characteristics of the active cells attached to electrodes (Khan *et al.*, 2013).

Gorby *et al.*, (2006) exclude the possibility that the current was being carried by ionic conduction from the possible presence of water and dissolved ions by investigating the

distance reliance of the current at various locations over bacterial nanowires appendages. The same kind of work was done by El-Naggar *et al.*, (2010) in the nanowires of *S. oneidensis* which were found to be electrically conductive along micrometer-length scales with electron transport rates up to $10^9/s$ at 100 mV of applied potentiality and a calculated resistivity on the order of 1Ω . Leung *et al.*, (2013) reported that the *S. oneidensis* nanowires show electronic behavior with a field effect mobility on the order of $10^{-1} \text{cm}^2/(\text{Vs})$. The trend shows an increase in electron transfer for bacterial nanowires development. This shows that the bacterial nanowires oxidation generates an increasing number of electrons, which is transferred extracellular to the electrode (Ramasamy *et al.*, 2008, Dominguez-Benetton *et al.*, 2012, Malvankar *et al.*, 2012, Khan *et al.*, 2013). Extensive of the voltage moves the fermi level away from this specific condition, resulting in lower conductance or even a reduction of current comparable to blank sample. As fitted the equivalent circuit with the bacterial nanowire thin film exhibited the small polarization resistance as found to be 4457.0. The CPE-T value was found to be $6.26 \mu\text{Fcm}^{-2}$. As described in the earlier section, bacterial nanowires thin film possessed small polarization resistance and large double layer capacitance in comparison to the blank electrode, suggesting that bacterial nanowire accelerated the EET. This result showed that the electrochemical conductivity of the bacterial nanowires thin film was increased in comparison to the blank electrode.

The conductive flagella provide the opportunity to extend electron transfer capabilities well beyond the outer surface of the cells. These results show that the flagella of *P. aeruginosa* are highly conductive. The flagella are anchored in the periplasm and outer

membrane of cells, thus offering the possibility that flagella accept electrons from periplasmic and/or outer membrane electron transfer proteins.

5.4 Metal oxide nanoparticles synthesis

The average length and diameter of the separated bacterial nanowires were to be 16 nm and 30 nm respectively. In particular, the metal oxide nanoparticles of less than 10 nm size would be more compatible for the fabrication of the nanocomposite with bacterial nanowires. Hence, metal oxide nanoparticles of copper oxide (CuO), nickel oxide (NiO) and zinc oxide (ZnO) were synthesized by chemical method. As prepared metal oxide nanoparticles and metal oxide coated bacterial nanowires were characterized by physiochemical and electrochemical techniques, which are discussed below.

5.4.1 UV-visible spectrophotometer studies

An absorption band was observed at around 275 nm for the CuO nanoparticles (Fig. 12). The obtained absorption peaks at 275 nm is attributed to the formation of CuO nanoparticles (Topnani *et al.*, 2009). The absorption peak is probably related to the electronic transition taking place; from valence band to the conduction band due to the quantum size of particle (Yin *et al.*, 2005). A weak, broad peak centered at ~450 nm is attributed to the band gap transition of CuO present at the surface of the nanocrystal (Wang *et al.*, 2009). The sharp absorpance peak leads to an decrease in particle size due to high surface energy of smaller particles with controlled shape, supporting the formation of uniform-particle in CuO nano powder (Topnani *et al.*, 2009). It is concluded that the broadness of the absorption edge

shift to lower wavelength due to the small particle size with wide distribution, with the help of tuning the synthetic methodology (Topnani *et al.*, 2009).

The UV–visible absorption peaks were observed about at 387 and 660 nm for the NiO nanoparticles (Fig. 13). The almost similar absorption peak was reported that the strong absorption in the investigated region was observed at wavelength about 353 nm (Alagiri *et al.*, 2012), which is fairly blue shifted from the absorption edge of bulk NiO nanoparticles. It was clearly evident from Barakat *et al.*, (2013) report that the UV-vis spectrum of the NiO nanoparticles is quite different from that starting complex, confirming the strong band that appeared at 355 nm was due to NiO nanoparticle. This strong absorption band is attributed to the electronic transition from the valence band to the conduction band in the NiO semiconductor (Salavati-Niasari *et al.*, 2009, Yang *et al.*, 2009).

The UV-visible absorption spectrum was obtained for the ZnO nanoparticles about at 400 nm in Fig. 14. Kumar *et al.*, (2012) also recorded similar strong absorption maximum below 400 nm. The absorption maximum of the ZnO nanoparticles was shifted to higher wavelengths. This red shift can be attributed to the agglomerations in the ZnO nanoparticles (Babita *et al.*, 2006). The good absorption of the ZnO nanoparticles in the UV region proves the applicability of this product in such electrochemical and optical application (Kumar *et al.*, 2012).

5.4.2 FT-IR spectral studies

Phiwdang *et al.*, (2013) were reported noticeable peaks at 525 and 584 cm^{-1} which were attributed to Cu–O stretching modes. The absorption peaks for CuO nanoparticles in the range of 1395 and 1564 cm^{-1} that may be assigned to O –H bending vibrations combined

with copper atoms (Dubal *et al.*, 2010). The wide range peaks about at 3300–3700 cm^{-1} is due to hydroxyl groups, as demonstrated by Razavi and Loghman-Estarki, (2012). The similar result was observed an intense and wide peak center at 3440 cm^{-1} , which was assigned as H–OH stretching, and the peak at 1598 cm^{-1} corresponds to H–OH bending (Swarnkar *et al.*, 2011). Thus, FT-IR result suggests that the formation of copper oxide compound is obtained in this method accompanying the presence of C–O bonds corresponding to Cu–O stretching modes.

Fig. 16 shows the FT-IR spectra of NiO nanoparticles. Similar work was reported that the broad absorptions bands at 3430 and 1630 cm^{-1} are assigned to O–H stretching and bending modes of water molecules, respectively (Wu *et al.*, 2004, Alagiri *et al.*, 2012). Li *et al.*, (2008) observed that the absorption band around 1384 cm^{-1} can be ascribed to CO_3^{2-} ions the peak at 1114 cm^{-1} corresponds to stretching and bending vibrations of the intercalated C–O species. But a new peak is observed at 528 cm^{-1} , which correspond to Ni–O nanoparticles stretching mode (Song and Gao, 2008).

As depicted in Fig. 17, absorption bands at ~450–500 cm^{-1} , which were the stretching mode of ZnO. The weak band at 460 cm^{-1} was observed, which was the characteristic of metal–oxygen (M–O) vibration band (Nakamoto, 1978). Ghule *et al.*, (2006) also reported a weak broad band at 732 cm^{-1} might be due to the overlap of the bands at 710 and 750 cm^{-1} , which were characteristic of I_β and I_α phases of cellulose. This possibly indicates their presence as a mixture in the composition of paper (Liu *et al.*, 2005). The bands in 1300 cm^{-1} and 1600 cm^{-1} were associated with C–O and C–H vibrations of cellulose (Liu *et al.*, 2005).

5.4.3 XRD pattern studies

The obtained 2θ value corresponds to the crystal planes of crystalline CuO, supporting all reflections can be indexed to correlate the monoclinic phase of CuO (Wang *et al.*, 2009). Our XRD results thus confirm synthesis of pure and well crystalline CuO nanoparticles without any impurity. The obtained results were well consistent with the previously reported literature (Langford and Louer, 1991, Abaker *et al.*, 2011). The rate of particle aggregation is a major factor that controls the morphology and structure (crystalline) of the final product (Huff *et al.*, 1993). Literature also indicates that with increase in calcination temperature the crystallinity of the particle increases as well as the surface area decreases (Vidyasagar *et al.*, 2012). Variation in calcination temperature brought about changes in the crystallinity (Mahato *et al.*, 2011).

The XRD diffraction pattern of the NiO nanoparticles exhibited well defined and resolved broad peaks indicating the crystalline nature of the NiO nanoparticles, as shown in Fig. 18. In addition, the XRD pattern agreed with Motlagh *et al.*, (2011) for the nickel oxide nanoparticles. The solvent used plays an important role in deciding the reduction path and the nature of the product. In the previous report, Ni²⁺ ions to Ni (0) in reverse micellar system were employed to prepare the nickel nanoparticles (Zhang *et al.*, 2015).

The XRD pattern of the ZnO nanoparticles is shown in Fig. 19. There were no other peaks observed for the ZnO nanoparticles, confirming the high purity of the synthesized product of ZnO. The intensity of the peaks increases with calcinations temperature, indicating increased crystallinity of the ZnO. XRD pattern indicates the formation of hexagonal wurtzite phase of ZnO which is in agreement with the electron diffraction results. The peak broadening in the XRD pattern clearly indicates that small nanocrystals are present

in the samples. There is no evidence of bulk remnant materials and impurity. Nine peaks appear at different 2θ values as shown in the Fig. 19. The sharp diffraction peaks indicate the good crystallinity of the prepared particles (Bindu and Thomas, 2014). Similar X-ray diffraction pattern were reported by Chen *et al.*, (2011), Pung *et al.*, (2012).

5.4.4 AFM studies

In order to examine the formation of nanoparticles, the surface analysis of CuO nanoparticles on the glass substrate was examined by AFM (Fig. 21 a-c). From the Fig. 21, it is visible that single CuO spherical nanoparticle was formed in all cases. The results show that morphology of the all CuO nanoparticles were similar. In Fig. 21 (a) single as well as accumulated particles are clearly visible.

AFM images were obtained by the measurement of the interaction forces between the tip and the sample surface. AFM provided cross-sectional 2D and 3D images of the NiO nanoparticles (Fig. 22). Both techniques revealed the smooth characteristic of the film of NiO nanoparticles. The NiO nanoparticles morphology was also spherical. This image indicate that the size of NiO nanoparticles were similar in all the spots of the thin film (Fig. 22 a).

The ZnO nanoparticles were deposited on the substrate and were examined by AFM, as shown in Fig. 23 a-c. It can be inferred from this 2D image that the ZnO nanoparticles are very well coated on the substrate and that the distribution of the nanoparticles on the surface was almost uniform with good accumulation. The ZnO nanoparticles morphology was also spherical in nature and the distribution of the size was similar.

The roughness of the metal oxide nanoparticles (CuO, NiO and ZnO) was measured by AFM. The height of particles indicates that the particle size is in good agreement which

was showed in Fig. 21c, 22c, 23c (Klapetek *et al.*, 2011). This result indicates that the growth of larger grains with increasing thickness leads to an increase in the surface roughness (Hammoodi *et al.*, 2014). Moreover, the maximum roughness peak of CuO nanoparticles was at the height of 1.1 nm (Fig. 21 c) and it is lower than NiO and ZnO nanoparticles roughness maximum peak. The maximum roughness peak of NiO nanoparticles and ZnO nanoparticles was 1.4 (Fig. 22 c) and 1.8 respectively (Fig. 23 c). An increase in the nanoparticles roughness height automatically leads to increase in the roughness valley depth.

5.4.5 SEM studies

The nanoparticle size and distribution depends on the effect of time, synthetic methodology, morphology, and the nature of molecular precursor used. The SEM images are displayed in Fig. 24 a-d for the CuO nanoparticles. The present chemical reduction methodology suggested that the formation of well-dispersed spherical and regular polyhedron shape for the CuO nanoparticles, with clear porosity, as displayed in Fig. 24. The primary particles appeared in the form of agglomerates, ranging from submicron to few microns in size. Depending on experimental conditions, an ultrafine, uniform, and aggregated spherical crystallite particle, with modal diameters around 10 nm was observed (Topnani *et al.*, 2009). At the nanosized scale, the individual nanoparticle has limited aggregation. In case of Cu₂O, the individual particle was nearly sphere shape with aggregation (Du *et al.*, 2009). In case of CuO nanoparticles limited crystallinity with octahedral shape was observed, thereby supporting the presence of limited aggregation (Liu *et al.*, 2006, Wang *et al.*, 2009, Topnani *et al.*, 2009).

The SEM image of the NiO nanoparticles illustrated in Fig. 25, clearly shows that the shape and size of particles are quite different from the precursor complex. It can be seen that the product was formed from extremely fine semi-spherical particles that were loosely aggregated. No characteristic morphology of the complex is observed, indicating the complete decomposition into the extremely fine spherical shape of particles (Barakat *et al.*, 2013). The NiO nanoparticles show the diameter of the shafts becomes smaller along the length of the particles and finally forms nanoscale tips (Yuan *et al.*, 2006).

The morphology of the synthesized ZnO nanoparticles was analyzed using SEM and is shown in Fig. 26. The SEM image clearly demonstrates the formation of spherical ZnO nanoparticles. In the present work it appears that the aggregation is the dominant mechanism which occurred during the crystallization of network leading to ZnO particles. Du *et al.*, (2014) reported a new reaction to synthesize ZnO nanoparticles with nearly uniform, spherical morphologies and controlled the size range from 25–100 nm via esterification of zinc acetate and ethanol under solvothermal reaction conditions. The observed particles consisted not only of single primary nanocrystallites but of secondary ZnO particles formed by fusion of several primary crystallites. Thus, aggregations of ZnO primary crystallites constructed the larger polycrystalline particles observed in SEM analysis (Rani *et al.*, 2008, Tan *et al.*, 2012). Moreover, spherical shaped nano crystallites might be in a stable form, as indicated by SEM analysis.

5.4.6 HR-TEM studies

The morphology of the synthesized CuO nanoparticles was further characterized by HR-TEM technique, as shown in Fig. 27. These images demonstrate that the CuO

nanoparticles were mostly spherical in shape and not agglomerated. The different magnification of HR-TEM images exhibited a clear dispersion of small size CuO nanoparticles. As can be displayed in Fig. 27, the CuO nanoparticles were highly mono-dispersed nature and the average particle size was determined to be 3.8 nm. HR-TEM revealed that the size of the NiO nanoparticles was in the range of 2.14 to 3.90 nm.

Fig. 28 shows the HR-TEM images of the NiO nanoparticles with different magnifications. It is seen that the nanocrystals are more or less cubic and the average particle size was 4 nm. The isotropic nature of NiO nanoparticles were prepared by chemical method. The high-resolution TEM images suggested that the very small size of NiO nanoparticles were obtained. The NiO nanoparticles obtained in the size range of 1.71 nm to 3.66 nm, explaining that the quantum size of NiO nanoparticles were prepared for making nanocomposite with bacterial nanowires. The HR-TEM images reveal that there is no agglomeration in the particles of the nanocrystals.

The HR-TEM images of ZnO nanoparticles are displayed in Fig. 29, which were clearly showed that the average size of the ZnO nanoparticles was estimated to be 3.1 nm and there was an inclination forming at thin planar structures than the spherical structures. There is an interesting fact that not all the particles of the HR-TEM images are in physical contact, but was separated from each other by uniform interparticle distance. An important and effective benefit obtained by this method is that the nanoparticles synthesized were stable in solution. This character makes it more potent compared to other biological methods which are currently in use (Nirmala *et al.*, 2013).

5.5 Metal oxide nanoparticles coated bacterial nanowires

The prepared metal oxides of CuO, NiO and ZnO nanoparticles were mostly spherical in shape and the average size was less than 4 nm. The small size of nanoparticles was used to prepare the nanocomposite of metal oxide nanoparticles coated on bacterial nanowires. The metal oxide nanoparticles were coated over ultrathin bacterial nanowires that are randomly oriented, creating a spherical shell full of bacterial nanowires. The thickness of bacterial nanowires was increased after metal oxide nanoparticles dispersion. It should be noted that the condition used here produces bacterial nanowires made of thin sheets covering the metal oxide nanoparticles. The metal nanoparticles coated bacterial nanowire is no earlier reported in the literature. It is first of its kind to study the nanocomposite of semiconductor metal oxide nanoparticles coated bacterial nanowires. The nanoparticles were made of using the cross linkage of the semiconductor metals and bacterial nanowires and this has an advantage of having significantly larger surface area, which was desirable for electrochemical applications.

5.6 Electrochemical studies of the metal coated bacterial nanowires

5.6.1 CV studies

To understand the electrochemical conductive properties and the conversion mechanism of the CuO nanoparticles coated bacterial nanowires, CV was employed to measure the response of the nanocomposite electrodes using a three-electrode electrochemical cell. Fig. 33b shows typical CV curves of the CuO nanoparticles coated bacterial nanowires electrode at a slow scan rate of 20 mVs^{-1} in a wide voltage range of 0.0 to 1.0 V. The CuO nanoparticles coated bacterial nanowires thin film electrode displayed

strong anodic peak about at ~ 0.2 V. The observed anodic peak was obtained by the oxidation of CuO. In addition, a minor oxidation peak at 0.26 V was observed during the anodic scan. This peak may be attributed to the oxidation of under layer of CuO on the bacterial nanowires that formed by nanocomposite of CuO nanoparticles with bacterial nanowires (Yuan *et al.*, 2011, Li *et al.*, 2014). The CuO nanoparticle coated bacterial nanowires electrode showed obviously enhanced anodic current as compared with that of bacterial nanowires electrode. This enhancement is attributed to the high electrochemical activity of CuO nanoparticles coated bacterial nanowires and the increment in the specific surface area of the electrode (Donders *et al.*, 2009). This finding also revealed that the mass transfer phenomenon in the double layer region of the electrodes was mainly diffusion-controlled (Yu *et al.*, 2008). As compared with the bacterial nanowires electrode, the decoration of the bacterial nanowires with CuO nanoparticles further increased the effective surface area of the CuO nanoparticles coated bacterial nanowires electrode much times leading to significantly enhanced electrochemical activity of the CuO nanoparticles coated bacterial nanowires electrode.

Fig. 34 a and b shows the CV of bacterial nanowires and NiO nanoparticles coated on the bacterial nanowires in 0.1M H₂SO₄, at a scan rate of 20 mVs⁻¹. The CVs for the bacterial nanowires and NiO nanoparticles coated bacterial nanowires in the same experimental conditions and is shown in Fig. 34 a and b signifying electrode property was enhanced. Differences in peak current ranges between voltammograms from the same electrolyte were attributed to the major differences in NiO nanoparticles loaded bacterial nanowires loading on the GC electrode surface. During the following process, the peak is located at 0.36 V, which can be attributed to the oxidation reaction of NiO nanoparticles on the bacterial

nanowires (Yuan *et al.*, 2010, Tao *et al.*, 2012). In the subsequent two cycles, the main cathodic peak potentials shift to 0.21 V. The CV curves were stable and almost overlapped, indicating the good reaction for NiO nanoparticles coated bacterial nanowires electrode.

These results were in line with the previous work that explains the behavior of nanoparticles as the facilitators of electron transfer (Bard *et al.*, 2001, Zhao *et al.*, 2001). Hence, NiO nanoparticles have been reported to generate a high capacitance due to its rapid and reversible proton intercalation in the lattice of nanocomposite (Bard *et al.*, 2001, Zhao *et al.*, 2001). The curve shapes from both electrodes present characteristics that were typical of an ideal electrochemical activity of the nanocomposite which were reliable with the results of the CV curves.

In Fig. 35 a and b the CV of the bacterial nanowires and ZnO nanoparticles coated bacterial nanowires thin film electrode recorded at a scan rate of 20 mVs^{-1} . The background current was enhanced and redox peaks were diminished by the dispersion of ZnO nanoparticle the surface of the bacterial nanowires, as displayed in Fig. 35 a. From the CV measurement, the nature of double-layer charge was stored at the electrode-electrolyte interfaces, which can be gathered (Kumar *et al.*, 2012, Shukur and Kadir, 2015). Previous results have shown that ZnO coated electrodes perform better irrespective of the applied potential or salt concentration (Laxman *et al.*, 2014, Laxman *et al.*, 2015) and its efficiency can be further improved by controlling the length, density and morphology of the ZnO nanoparticles (Myint and Dutta, 2012, Laxman *et al.*, 2014, Myint *et al.*, 2014). Upon coating ZnO nanoparticles with bacterial nanowires, the role of surface area in increasing the desalting capacity of the composite electrode was ruled out based on surface area measurements. The pore structure of ZnO nanoparticles that which can be attached as an

overlapping layers at the bacterial nanowires (García-Quismondo *et al.*, 2013), which is overcome by the well distributed field around the bacterial nanowires as described in previous works (Laxman *et al.*, 2015). Hence, the improvement in ZnO nanoparticles adsorption efficiency with bacterial nanowires could be attributed to a combination of increased electrochemical activity combined with the electrode surface.

5.6.2 LSV studies

LSV studies of the bacterial nanowires and CuO nanoparticles coated bacterial nanowires thin film electrode are shown in Fig. 36 a and b. In the Fig. 36 a, the small anodic peak was obtained for the bacterial nanowire electrode, which may correspond to the oxidation properties of the bacterial nanowire. The CuO nanoparticles coated on the bacterial nanowires exhibited a well-defined anodic peak about 0.2 V, corresponding to the oxidation of CuO nanoparticles. In the LSV scan, a greater amount of CuO nanoparticles was oxidized over the bacterial nanowires electrode and provided a well-defined anodic peak. The layering of CuO nanoparticles on the bacterial nanowires results correspond to the formation of metal oxide / protein layer electrode surface lead to increase in the capacitance of the electrode (Kauffman *et al.*, 2011). The result compared well those with the previous reports with respect to voltammetry (Kang *et al.*, 2008, Lin *et al.*, 2012)

The LSV results of bacterial nanowires and NiO nanoparticles coated bacterial nanowires are shown in Fig. 37 a and b. At 0.57 V, the thin film of NiO nanoparticles coated bacterial nanowires electrodes showed a higher anodic current compared to the bacterial nanowires thin film electrode at 0.4 V, and this revealed that the NiO nanoparticles coated bacterial nanowires electrode might be performed the good electrochemical conductive

properties. The good performance should be ascribed to the high content of electrochemical activity on the NiO nanoparticles coated bacterial nanowires electrode. The NiO nanoparticles coated bacterial nanowires electrode showed a much higher anodic current compared to the bacterial nanowires electrodes, suggesting that the porous surface of NiO nanoparticles should be responsible for the current enhancement. However, electrochemical property of the bacterial nanowires was enhanced by the mono-dispersion of the NiO nanoparticles on the bacterial nanowires surface. These results further indicate that advanced role of the coated NiO nanoparticles into the bacterial nanowires layers to enhance electrochemical activity (Lin *et al.*, 2012, Zhang *et al.*, 2015).

There is a linear relationship between the values of the conductance which indicates the number of free current carriers in the semiconductor. And it is well established that LSV can be used to investigate the electrochemical performance of semiconductive films (Zhang *et al.*, 2006). The conductivity increased linearly with the applied potential could represent the electrochemical oxidation of semi conductive films (Jiang *et al.*, 2001, Zhang *et al.*, 2015). In addition, electrochemical method is usually used to characterize charge separation of the films (Leng *et al.*, 2005, Zheng *et al.*, 2008, Zhang *et al.*, 2015). LSV voltammograms of the bacterial nanowires and ZnO nanoparticles coated bacterial nanowires measured in Fig. 38 a and b. It is clearly seen that the anodic potential of ZnO nanoparticles coated bacterial nanowires (Fig. 38b) is much higher than that of bacterial nanowires (Fig. 38a) on the GC electrode which revealed that there were a larger number of free electron transport carriers in ZnO nanoparticles coated bacterial nanowires (Zheng *et al.*, 2008, Zhou *et al.*, 2011). Here the anodic peak represents the electrochemical oxidation in nanowires which explicates that there are more less-positive potential in ZnO

nanoparticles coated bacterial nanowires (Zhang *et al.*, 2015). Furthermore, the ZnO nanoparticles coated bacterial nanowires showed a little broad anodic peak with less negative shift, as shown in Fig. 38b. The ZnO nanoparticles coated bacterial nanowires demonstrated a much higher electrochemical capacitance to the bacterial nanowires, signifying that the surface of ZnO nanoparticles should be accountable for the electrochemical activity, as well as the conductivity enhancement. Hence, the electrochemical capacitance and conductivity enhancement of the bacterial nanowires was possible due to the dispersion of the ZnO nanoparticles.

5.6.3 EIS studies

The main objective of the EIS experiments is to knowing the effect of CuO nanoparticle coated on the bacterial nanowires at the interfacial properties of the electrodes. The Nyquist plot of the bacterial nanowires and CuO nanoparticles coated bacterial nanowires electrodes is shown in Fig. 39 a and b. R_p value of the bacterial nanowires electrode was much higher than that of CuO nanoparticles coated bacterial nanowires electrode. This was due to the increase of electrical conductivity of the hetero structures (CuO nanoparticles coated bacterial nanowires) after CuO nanoparticles were deposited onto the bacterial nanowires (Lai *et al.*, 2012). The polarization resistance of the CuO nanoparticles coated bacterial nanowires electrode was two-times smaller than that of the bacterial nanowires electrode, indicating that the electrochemical resistivity of the electrode interface was significantly improved. This finding in Fig. 39b increase of electrochemical conductance of the CuO nanoparticles coated bacterial nanowire electrode was attributed to the good electrical resistance efficiency of CuO nanoparticles coated bacterial nanowires and

the large effective surface area of the hetero structure electrode (Li *et al.*, 2014). The polarization resistance was changed about two times with increasing the double layer capacitance in the presence of metal oxide nanoparticles; however, the effect of the nanoparticles in providing suitable electrochemical activity for observing the direct electron transfer in CuO nanoparticles in addition to preserving its natural structure of bacterial nanowires and electrochemical polarization resistance should be considered.

EIS was investigated for the bacterial nanowires and NiO nanoparticles coated bacterial nanowires electrodes by applying constant applied potential of 0.25V and are shown in Fig. 40 a and b. Apparently, the diameter of the semicircle for the NiO nanoparticles coated bacterial nanowires electrode was decreased after dispersion on the surface of the bacterial nanowires, indicating the smaller polarization (Hwang *et al.*, 2012, Kim *et al.*, 2012, Kim *et al.*, 2013). Thereafter, the impedance test at the end of cycle indicated that bacterial nanowires without NiO nanoparticles show an increased polarization resistance (Fig. 40a). It is possible that conduction inside the layers and charge transfer at the electrode/electrolyte interface is delayed by the increase in internal defects and isolated active regions due to the volume change and subsequent microstructural failure during cycle (Huang *et al.*, 2007, Kang *et al.*, 2008, Liu and Zhang, 2009, Hwang *et al.*, 2012, Kim *et al.*, 2013).

In contrast, the semicircle of Nyquist plot, corresponding to the NiO nanoparticle coated bacterial nanowires, shows extremely low polarization resistance in Fig. 40 b. This behavior indicates that the NiO nanoparticles deposited on the bacterial nanowires surface were favorable for the formation of thicker film (Liu and Zhang, 2009, Kim *et al.*, 2013). The NiO nanoparticles coated bacterial nanowires structure appears to reach a stabilized

state, which is consistent with the observed extra capacity of the NiO nanoparticles coated bacterial nanowires electrode in Fig. 40 b. In addition, to confirm the structural integrity of the prepared films, the evolution of the morphology and structure of bacterial nanowires and NiO nanoparticles coated bacterial nanowires were investigated as shown in Fig.40.

The above results were consistent with the development of a synergistic effect as the result of the NiO deposited on bacterial nanowires, which leads to an improved electrochemical performance. The biological conductive material of bacterial nanowires and interconnected NiO nanoparticles facilitate continuous conductive pathways for electrons and provide a sufficient electrolyte contact area, resulting in the formation of a stable layer. Moreover, due to the accommodation of the pulverization of active materials during the cycling process, the structural integrity of the electrodeposited NiO nanoparticles coated bacterial nanowires can be effectively maintained.

EIS was performed to evaluate the polarization resistance of ZnO nanoparticles coated bacterial nanowires. Clearly, impedance spectrum was composed of two different segments, charge-transfer limited dependent semicircle (high frequency region) and the warburg diffusion limited straight line (low frequency region). The ohmic and polarization resistances were shown in the high-frequency intercept with the vertical axis and the diameter of a semicircle in the low-frequency range, respectively (Xiao *et al.*, 2012, Mehdinia *et al.*, 2014). Fig. 41 a and b the EIS spectra of the bacterial nanowires and ZnO bacterial nanowires electrodes were presented. The parameters of the polarization resistances obtained by fitting the impedance spectra (Leng *et al.*, 2005) are shown in Table 1. As shown in Fig. 41, the circular radius and calculated the impedance components of the ZnO coated bacterial nanowires electrode were dramatically smaller than that of bacterial

nanowires electrode, indicating that the ZnO nanoparticles coated bacterial nanowires nanocomposite electrode possess lower polarization resistance with higher capacitance than the bacterial nanowires thin film electrode. Moreover, the result obtained by EIS shows that bacterial nanowires has a larger charge transfer resistant constant compared with ZnO nanoparticles coated bacterial nanowires, which means higher conductivity efficiency of ZnO coated bacterial nanowires. It could be explained that the recombination of conductivity effectively enhanced by the incorporation of ZnO nanoparticles, resulting in better electrochemical conductivity in the ZnO coated bacterial nanowires. Thus, the electrochemical conductivity enhancement of ZnO nanoparticles coated bacterial nanowires are also evidently improved electrical conductivity. Furthermore, Fig. 41b, also shows that the polarization resistance of the ZnO nanoparticles coated bacterial nanowires was lower than that of bacterial nanowires electrode, which means a lower charge transfer resistance in ZnO nanoparticles coated bacterial nanowires, which might be responsible for the increase in the electrical conductivity.