

**Improving the Performance of Graphite Anode in a Microbial Fuel Cell *via* PANI
Encapsulated MnO₂/NiO Nanocomposite Modification for Efficient Power
Generation and Biodegradation**



Yilkal Dessie Sintayehu

**A Dissertation Submitted to
The Department of Applied Chemistry
School of Applied Natural Sciences**

**Presented in Partial Fulfillment of the Requirement for the Degree of Doctor of
Philosophy in Materials Chemistry**

**Office of Graduate Studies
Adama Science and Technology University**

September, 2021

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Co-Supervisor: Rajalakshmanan Eswaramoorthy (Assoc. Prof., Ph.D.)

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We, the undersigned, members of the Board of Examiners of the final open defense by Yilkal Dessie Sintayehu have read and evaluated his thesis entitled **"Improving the Performance of Graphite Anode in a Microbial Fuel Cell *via* PANI Encapsulated MnO₂/NiO Nanocomposite Modification for Efficient Power Generation and Biodegradation"** and examined the candidate. This is, therefore, to certify that the dissertation has been accepted in partial fulfillment of the requirement of the Degree of Doctor of Philosophy in Materials Chemistry.

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DECLARATION

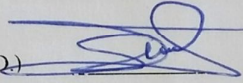
I hereby declare that this PhD Dissertation is my original work and has not been presented for a degree in any other university, and all sources of material used for this dissertation have been duly acknowledged.

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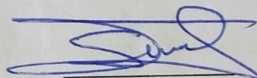
APPROVAL OF SUPERVISORS

To: Department of Applied Chemistry

Subject: Dissertation Submission

This is to certify that the dissertation entitled **“Improving the Performance of Graphite Anode in a Microbial Fuel Cell via PANI Encapsulated MnO₂/NiO Nanocomposite Modification for Efficient Power Generation and Biodegradation”** submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Materials Chemistry, the Graduate program of the Department of Applied Chemistry, and has been carried out by Yilkal Dessie, ID. No GSR/022/10, under our supervision. Therefore, we recommend that the student has fulfilled the requirements and hence hereby he can submit the dissertation to the department.

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LIST OF ACRONYMS AND ABBREVIATIONS

ATP	Adenosine triphosphate
AC	Alternating current
APS	Ammonium per sulfate
I_{pa}	Anodic peak current
E_{pa}	Anodic peak potential
AFM	Atomic force microscopy
BES	Bioelectrochemical systems
BOD	Biological oxygen demand
I_{pc}	Cathodic peak current
E_{pc}	Cathodic peak potential
R_{ct}	Charge transfer resistance
COD	Chemical oxygen demand
CCV	Closed circuit voltage
CWs	Constructed wetlands
CE	Coulombic efficiency
CryOD	Crystallography Open Database
CV	Cyclic voltammetry
DE	Decolorization efficiency
DNA	Deoxyribonucleic acid
DoE	Design of experiments
DSC	Differential scanning calorimeter
DTA	Differential thermal analysis
C_{dl}	Double layer capacitance
EAM	Electroactive microorganisms
EIS	Electrochemical impedance spectroscopy
EAB	Electrochemically active bacteria
E_g	Energy band gap
EDX	Energy dispersive X-ray analysis
FTIR	Fourier transforms infrared spectroscopy

FWHM	Full width at half maximum
T _g	Glass transition temperature
IUPAC	International Union of Pure and Applied Chemistry
R _{ku}	Kurtosis coefficient
LED	Light emitting diode
MFM	Magnetic force microscopy
R _a	Mean roughness
R _q	Mean square roughness
MR	Methyl red
MFC	Microbial fuel cell
NMs	Nanomaterials
NPs	Nanoparticles
NADH	Nicotinamide adenine dinucleotide (NAD) + hydrogen (H)
OFAT	One factor at a time
V _{OC}	Open circuit voltage
ORP	Oxidation-reduction potential
ORR	Oxygen reduction reaction
PGE	Pencil graphite electrode
PBS	Phosphate buffer solution
PANI	Polyaniline
PPy	Polypyrrole
PVA	Polyvinyl alcohol
PEM	Proton exchange membrane
RE	Reference electrode
RSM	Response surface methodology
RVC	Reticulated vitreous carbon
SCE	Saturated calomel electrode
SEM	Scanning electron microscope
SNOM	Scanning near field optical microscopy
SPE	Scanning probe microscopy

SThM	Scanning thermal microscopy
Ag/AgCl	Silver/Silver chloride electrode
R_s	Solution resistance
SHE	Standard hydrogen electrode
R_{sk}	Surface skewness
TGA	Thermogravimetric analysis
3D	Three dimensional
TMOs	Transition metal oxides
2D	Two dimensional
2FI	Two factor interaction
UV-Vis	Ultraviolet visible spectroscopy
Z_w	Warburg impedance
WE	Working electrode
XRD	X-ray diffractometer

ABSTRACT

The energy crises caused by continual usage of non-renewable energy sources along with environmental pollution are causing a detrimental effect on ecosystems and human health. The growing demand for energy, rapid urbanization, and increasing industrialization have an enormous impact on environment pollution. Waste directly discharged from various industries have been affecting soil, groundwater, and rivers. To address such problems, microbial fuel cells (MFCs) are an innovative bioelectrochemical system for those waste conversion into useful form of energy and for clean water demands. Among MFC components, the anode electrode plays a vital role in energy generation and simultaneous pollutant removal. In this study, improving electrochemical property of anode with biosynthesized MnO_2 based composite catalysts were helped to enhance microbial attachment to form conductive biofilm, substrate degradation and extracellular electron transfer rate to enhance efficiently. The green synthesis of $\alpha\text{-MnO}_2$ nanoparticles (NPs) and $\alpha\text{-MnO}_2/\text{NiO}$ bimetallic oxides were achieved in the presence of *Vernonia amygdalina* leaf extract. The biosynthesized $\alpha\text{-MnO}_2$ NPs and $\alpha\text{-MnO}_2/\text{NiO}$ bimetallic oxides were optimized by Optimal Coordinate Exchange Design model. The effects of each factors on absorption responses were analyzed by ANOVA using second and first degree polynomial equations. A very low p-values (< 0.0001), and reasonable regression coefficient values (coefficient $R^2 = 0.9790$) suggested that there is an effective correlation between experimental results and predicted values for $\alpha\text{-MnO}_2$ NPs while the good regression coefficient ($R^2 = 0.9873$) as well as adequacy of precision (16.8772) proved the good correlation between actual and predicted values for $\alpha\text{-MnO}_2/\text{NiO}$ bimetallic oxides NPs. Both binary $\alpha\text{-MnO}_2/\text{PANI}$ and ternary $\alpha\text{-MnO}_2/\text{NiO}/\text{PANI}$ nanocomposites were prepared by PANI encapsulating with $\alpha\text{-MnO}_2$ and $\alpha\text{-MnO}_2/\text{NiO}$ bimetallic oxide NMs by chemical polymerization technique. Physicochemical and structural analysis of the synthesized materials were characterized through UV-Vis spectroscopy, X-ray diffractometer (XRD), Fourier transform infrared (FTIR), thermogravimetric-differential thermal analysis (TGA-DTA), differential scanning calorimeter (DSC), scanning electron microscopy-energy dispersive X-ray analysis (SEM-EDX), Gwyddion software, cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS). The catalytic performance of bare PGE and NMs modified anodes were studied in glucose-fed-*Escherichia coli* based MFC. At steady state the performance elucidated by $\alpha\text{-MnO}_2/\text{PANI}$ composite modified bioanode was best and dominated with open circuit voltage (V_{oc}) having a value of 650.61 ± 10.11 mV. During non-steady state conditions, it was found that 506.96 ± 7.31 mW m^{-2} of power density was produced with $\alpha\text{-MnO}_2/\text{NiO}/\text{PANI}/\text{PGE}$. This was 7.71-fold higher in power density than unmodified PGE but its treatment efficiency in terms of COD and CE recorded by ternary composite was lower than that of binary $\alpha\text{-MnO}_2/\text{PANI}$ modified PGE. Thus 88.19% and 26.09% of COD and CE was determined using $\alpha\text{-MnO}_2/\text{PANI}$ based PGE at the same experimental conditions. After adding methyl red (MR) dye on anode chamber using this $\alpha\text{-MnO}_2/\text{PANI}$ binary composite modified PGE, a maximum DE of $95.57 \pm 2.26\%$, maximum power density of 820.02 ± 7.86 mW m^{-2} , current density 1990.34 mA m^{-2} with COD of $74.59 \pm 1.57\%$ were achieved using $\alpha\text{-MnO}_2/\text{PANI}/\text{PGE}$. To improve the biodegradation efficiency further, influences of MR were also studied. The achieved maximum power density and treatment efficiency by $\alpha\text{-MnO}_2/\text{PANI}$ modified bioanode catalyst was highest due to the better biocompatibility and efficient bioelectrocatalysis effect. Therefore, $\alpha\text{-MnO}_2/\text{PANI}/\text{PGE}$ provided as an alternative bioanode catalyst modifier and reusable to degrade glucose several times in the bioelectrochemical MFC device system towards simultaneous liquid waste treatment capability and renewable energy production.

1. INTRODUCTION

1.1. Background of the study

Water and energy securities have become a global challenges in recent years due to climate change, world population growth, increasing economic growth, and increasing material costs [1]. Consumption of excess energy from non-renewable fossil and hydrocarbon fuel based energy sources such as coal, oil, and natural gas causes environmental pollution due to the releasing of greenhouse gases [2]. Therefore resolving or improving one challenge could well improve the others. In this respect, finding a renewable and sustainable sources, instead of combusting fossil fuels, have been intensified to reduce both global energy crisis and climate change [3]. As a result, technologies relating to renewable energy sources such as solar, wind and biomass have progressed significantly [4]. Nevertheless, none of these can be competitive with mainstream fossil fuel based energy sources at this time, and this is why a new paradigm shift is required.

The rapidly growing microbial fuel cells (MFCs) technology is one example of a paradigm shift. MFCs are bioelectrochemical systems that convert energy contained in organic matter into electrical energy [5]. In addition to providing electricity generation, it also useful to treat wastewater *via* the action of biocatalysts [6]. MFC is an emerging technology with simple and cost-effective device to treat wastes with reducing energy crisis and global warming by producing green energy [7]. The source of this organic matters are from brewery, distillery, hospital, pharmaceutical, paper, wine, sewage sludge, mine drainage wastewater, or pulping wastewater [8]. For example, renewable energy from those waste discharges in the form of biofuels could be recovered as biogas by conventional means. But it only applied to treat wastes from concentrated sludge; however, such treatment could also result in the release of excess methane gas which is also a problem to the free atmosphere [9].

Research initiatives in Ethiopia would be focused on producing renewable energies from liquid waste streams [10] and societies in Ethiopia is energy dependent and need treated water for their life, household activities, and agricultural services at large, hence gradual interest is increasing to treat wastewater in the presence of electroactive microorganism (EAM). Since EAM is a biocatalyst. However, simultaneous bioelectricity production on those energy rich waste matters from various waste effluents is not yet practiced longer [11]. Therefore, in the next sections basics of bioremediation towards efficient bioelectricity

production and its treatment efficiency from various pollutants using nanoparticles (NPs) as a catalyst are discussed with the concept of MFC.

1.1.1. Bioremediation

Bioremediation is a process that uses either microorganisms or plant to detoxify contaminants present in soil, water or other environments [12]. Its process enhances the rate of degradation of contaminants by the action of microorganisms (either bacteria or fungi) with nutrients or carbon sources [12]. Bioremediation is an alternative and typically less expensive than equivalent physico-chemical methods due to its environmentally friendly nature, but using this technology alone has lack of effective contact between pollutant to microbes which causes slow reaction rate that affect the treatment efficiency [13]. To reduce this challenges, pollutants like azo dyes might act as a mediator during remediation process. However, pollutant selectivity, high time consumption, inappropriate and unachievable residual contaminant levels, and lower removal efficiency from waste influents are still the common drawbacks for conventional remediation's [14].

For the best bioremediation setup, microorganisms like gram negative bacteria due to their unique cell membrane nature can degrade hazardous organic wastes to their harmless products. These microbes transfer hazardous organic substances through reactions that take place as a part of their metabolic processes [12]. However, some halogenated contaminants like chlorinated organic or high aromatic hydrocarbons are resistant to such microbial attack during conventional bioremediation process. In most places of Ethiopia, halogenated based contaminates originated from domestic, industrial, and other agricultural streams are considered as a common waste disposals. When these wastes are directly discharged to soil, groundwater, and rivers which causes the deterioration of them. To show this, current research studies in this areas at about 94.85% [15], 65% [16], and 58% [14] maximum chemical oxygen demand (COD) removal efficiencies were succeeded from highly concentrated waste effluents using indigenous bacteria, anaerobic treatment, and hydroponics planted vetiver grass remediation methods, respectively. But their COD removal efficiencies were so poor due to low pollutant and biocatalyst interactions. Hence, the low-treatment efficiency as well as no conversion of COD into useful form of renewable bioelectricity are evidenced as enough research gaps to this dissertation study.

1.1.2. Microbial fuel cells

Devices to achieve bioremediations from the direct conversion of chemical energy that is stored in organic wastes into electrical energy through the mediation of exoelectrogenic bacteria have been emerged as a new paradigm [17]. In bioelectrochemical system the nature of electron transfers mechanisms, cell designation, electrode materials, membrane type, substrate, and microbes directly affects MFC performances [18] and its electrical circuit diagrams are shown in Figure 1 [7]. Biofilms that are found on anode surface hydrolyzed the complex organic matters which is present in the sludge into simpler molecules and further oxidized by electrochemically active microbes [19]. Organic wastes are injected into the anode bioreactor under anaerobic conditions while oxygen, atmospheric air or strong oxidant having with high reduction half potentials are applied into cathode chamber [20].

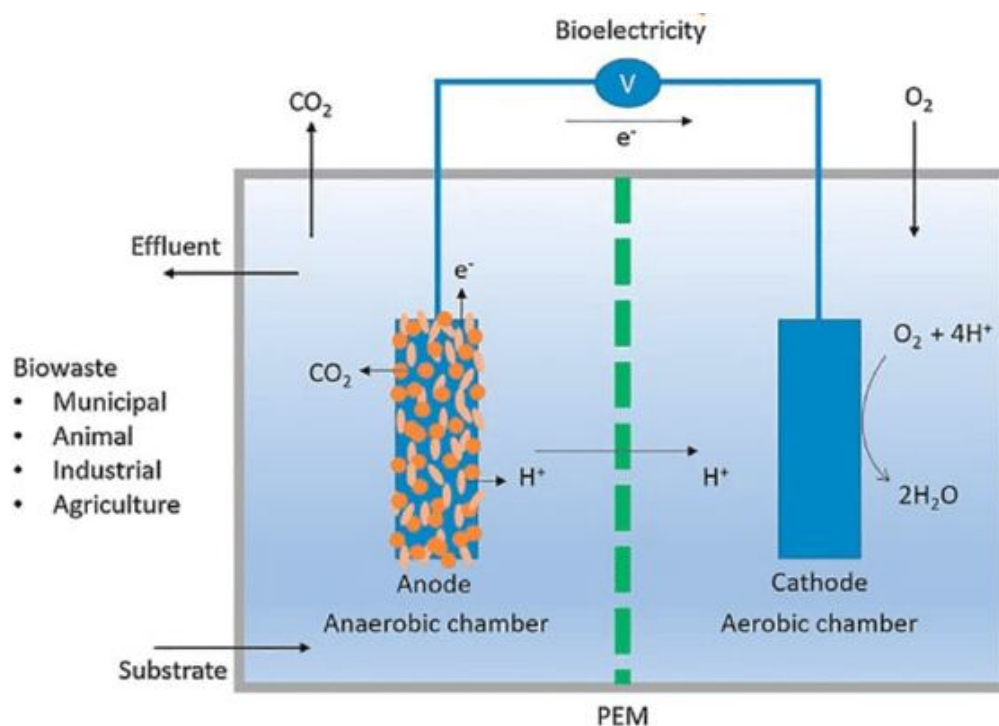


Figure 1. Graphical representation of microbial fuel cells [7].

In principle, a microbial community near the anode electrode uses organic substrates as fuels to produce different charge carriers like electrons and protons. Electrons collected at the anode are transported to the cathode by an external circuit while protons are transferred through the proton exchange membrane/or salt bridge internally. As a result, a potential difference is created between anode and cathode chamber due to dissimilar liquid solutions, and bioelectricity can be produced [21]. In MFC, electroactive bacteria which act as a

biocatalyst facilitates pollutant removal from real urban [22] and industrial [23] liquid waste discharges due to the presence of different organic substrates such as, glucose, acetate, and any other carbon source categories. From the industry side, this devices provide a promising approach in renewable energy recovery and waste treatment discharged from paper [24], brewery [14], tannery [6], and other sources of organic waste effluents industries [8].

The efficiency of MFC depends upon several factors from electrode type to its cell configurations [18]. Among these factors, the choice of suitable electrodes plays an important role in the development of best bioremediation performance with good energy conversion efficiency. High surface area, porosity, electrical conductivity, cost, accessibility, stability, biocompatible/microbial adhesion, durability, and non-toxic to bacteria are the most fundamental requirement for effective MFC device [25]. Because microbial biofilm growth, substrate oxidation, and electron transfer kinetics are highly dependent on anodic material for high MFC performance [26]. Creating a stable electrode surface in an optimum condition is also the other main challenging problem in MFC.

Next to stainless steel, conventional carbon materials are the other most common electrodes due to their relative low cost, biocompatibility, and chemical inertness but still produce low power density and have low waste removal efficiency [18]. Furthermore, they still produce weak biofilm formation due to the poor interaction with bacterial colony. Such problem is highly responsible for high internal charge transfer resistance formation and these could be explained in terms of polarization curve as shown in Figure 2. From the curve (A) activation, (B) Ohmic, and (C) concentration or mass transfer resistances (potential losses) are shown [27]. The presence of these factors limits power generation and decrease pollutant removal efficiencies, *i.e.*, limits MFC performances [28].

In MFC operations, the concentration polarization region, depletion of reactants due to mass-transfer resistance causes the output voltage to decrease very rapidly to zero [29]. The decoration of electrode with nanostructured material is the effective way to improve microorganism attachment due to the lower electron transfer resistance enhancement [30] and results for activation loss reduction near electrode surface [27]. Thermodynamically, NMs reduce an activation energy barrier for electron transfer between donor and acceptor while kinetically they increase reaction rates by addressing the rate-determining step. Therefore, the rate of electrochemical reaction on electrode surface without a catalyst would

be influenced by kinetics of electron transfer between analyte and electrode, the total available reactive surface area, and conductivity of the electrode [31].

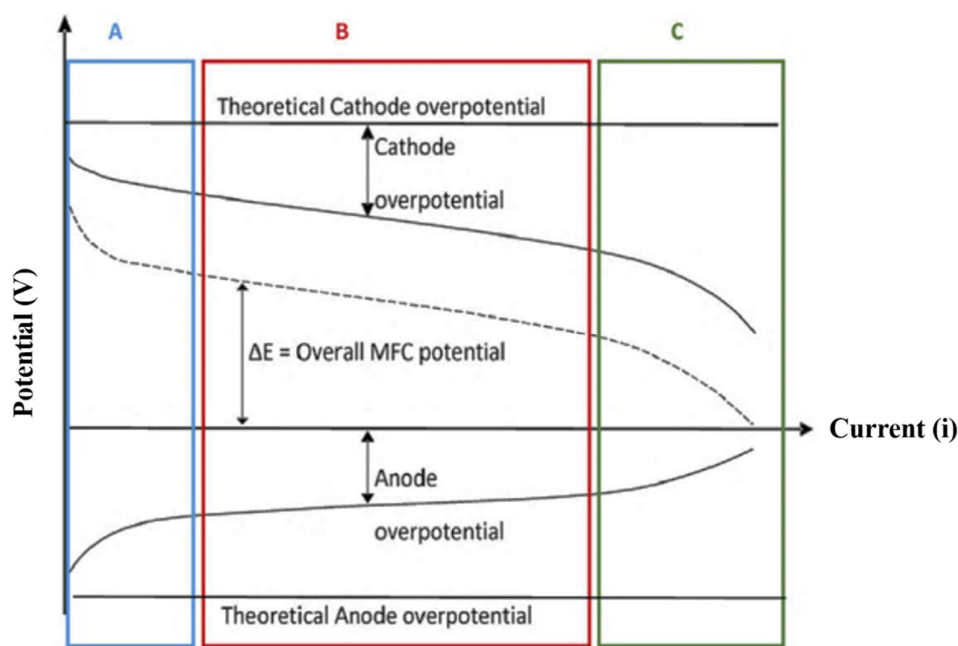


Figure 2. Fuel cell polarization curves and types of losses [27].

1.1.3. Nanomaterials

NMs from various types with diverse in sizes and compositions are much more attractive electrode modifiers due to their fast electron transfer capability during electrochemical oxidation/reduction reactions, compared to conventional bulk materials [32]. NMs from non-noble transition metal oxides (TMOs), conducting polymers, and their composites were assembled in MFC operation for wastewater treatment and green energy production due to their efficient catalytic activities, environment friendly, and inexpensiveness [33]. Their efficient electrocatalytic activities in the reduction-oxidation reaction enhanced better operational applications in MFC. Reports found that compositing could change both size and surface morphology of particle [34].

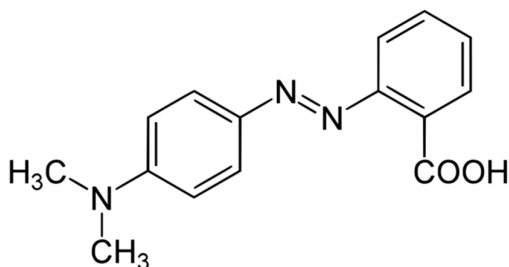
In the field of MFC, coating a metal electrode with polymer can be used to protect its surface from corruptions due to corrosion resistance behavior beyond improving its processing performance and electronic properties [35]. So, functionalization of metal oxide NMs with conducting polymers could also improve their electrical conductivity and environmental stability [25]. Conducting polymers contained from either PANI [36] or polypyrrole (PPy) [37] and metal oxides (MnO_2 and NiO) functional NMs were fabricated further as a

composite material based on standard bottom-up synthesis method to increase their catalytic activity, kinetics, redox stability, and active surface sites in MFC performances. Unloaded individual nanostructured materials sometimes but not always without a support suffer from a very low surface area which is responsible for low MFC performance. Therefore, in this dissertation study, MnO₂ based nanocomposites were used as a promising anode electrode modifier catalyst in MFC operational devices for those limitations.

1.1.4. Pollutants

Pollutants originating from organic, inorganic and others sources discharged from industrial effluents primarily affect the growth rate of plants and animal species including humans [38]. Such effluents are difficult to degrade effectively by conventional means of treatment strategy. Solving power deficiency from these huge organic waste effluents and keeping the environment from its pollution is the current priority strategy given by the academics across the globe. Thus due to the growing demand from environmental protection to sustainable development, MFC has attracted and have been given a widespread attention to treat them [39]. For example, producing a renewable energy from those waste effluents containing azo dyes have been proposed as an alternative approach to replace a non-renewable energy produced from fossil fuels [40]. Azo dyes having with C–N=N–C functional group which widely discharged from textile, paper, and cosmetic manufacturing are highly toxic to the environment. The continuous discharge of aromatic compound containing wastewater affect human health and living on the environment due to their toxicity and bioaccumulation [41].

Methyl red (MR) with chemical structure shown in Scheme 1 is thus considered as one of the most toxic dye containing a mono-azo bond which lacks sulfonate group in nature [42]. Physical, chemical, and biological methods have been carried out to treat a wastewater containing MR and other azo dye containing organic compounds [41,43]. However, all these methods are facing and suffering from several problems, such as required a lot of external energy, high operating and maintenance costs, enzyme instability during their operations, long decomposition periods with low decolorization efficiency, and further sludge production which may needs further overcome.



Scheme 1. Chemical structure of Methyl red (MR).

Quantitative information's from COD verified that, industries in Ethiopia are the sources of organic and inorganic waste pollutants. This might be due to the dominant overload of organic matter over the environment. However, its huge presence decreased the contact rate between the organic matter and electroactive microbial communities' results for decreasing in degradation efficiency for organic matter itself during conventional treatment. Brewery industries are the major polluting industries in Ethiopia. They generate large amounts of organic rich wastewater in to the free environments. Up to 95% and 98% COD removal efficiency of organic matter was treated by constructed wetlands (CWs) bioremediation method from brewery industry. CWs are promising treatment options for domestic and industrial wastewater through floating plant systems and numerous other configurations. The method is based on a combination of physical, chemical and biological treatment processes to remove organic matter, nitrogen, phosphorus and other substances. So, Other than Ethiopia, CWs technique is also common in Kenya, Tanzania, Thailand, and many other countries. During CWs process, climate and other local conditions influence wastewater characteristics, plant growth, and evaporation as well as the removal processes in the CW, particularly the microbial processes which may be stimulated by high temperatures. But at higher temperature a colony of microbes are not properly functioning [10]. Therefore, the poor understanding of interactions between electroactive microbial community and NPs needs in depth investigations where this study is focused.

1.2. Statement of the problem

The energy crises along with environmental pollution are causing a detrimental effect on ecosystems and human health. However, the ineffective organic waste degradation is considered a limiting factor for conventional treatment technology, and has prevented it from being widely adopted for the anaerobic treatment of chemical-industrial organic wastewater [38]. For such problems, there is a large gap between energy generation and treatment of wastewater for domestic wastewater in developing nations [44]. To date, few

optimized designs have managed to obtain even higher power densities up to $3\text{--}7\text{ W m}^{-2}$. However despite this progress, the power density of MFCs are still orders of magnitude lower than the chemical fuel cells (which are typically $> 10^4\text{ W m}^{-2}$ [45]. So far only a few on-site pilot-scale ($>10\text{ L}$) bioelectrochemical systems have been built and tested to attain success [46].

In Ethiopia, most of the industrial factories (*e.g.*, dairy and beverages) dispose their untreated toxic wastewater into the nearby rivers, lakes, and streams [47]. The level of wastewater management is low, even by sub-Saharan standards, due to a general lack of sanitation infrastructure, skill, and knowledge of wastewater treatment. A review assessed by Dejen Gashaye [48] showed that the wastewater treatment fraction was increase from 30% to 53% by 2020. 50-70% wastewater treatment capacity was done from wastewater flow in Addis Ababa to reduce the risk for farmers or consumers given the high absolute pollution load from the irrigated urban vegetable [48]. The release of untreated waste into the ecosystem affects the life of living things [49].

Recent work reported [50] that an internal resistance is a big factor that affects MFC performance, this factor is primarily raised from the nature and type of electrode materials having with low surface area, high electrode resistivity, low electrical conductivity, and inefficient chemical stability. Extended factor such as pH, temperature, substrate, circuit resistance, and electrode materials are also the other factors. Their presence would reduce part of the voltage and reduce power output during bioremediation. Coherently, there is a need to develop non-toxic superior catalytic material to avoid bacterial cell damaging, corrosion, and other degradation mechanisms of electrodes. Coatings them with conducting polymers encapsulated with nanostructured metal oxides offers an enhanced protection of bacterial cells from unfavorable factors [37]. Here are some of the most summarized factors which affect MFCs performance.

- Electrode cost and low performance of anode in MFC [50].
- The slow electrokinetic behavior found at the interface of substrate/microbial community/nanocomposites and electrode as well as unconditional biofilm accumulation inhibited power density and treatment efficiency [25].
- Substrate and its co-substrate concentration, low catalytic activity, as well as slow extracellular electron transfer between biocatalyst and NMs is a big problem and therefore need further investigations [51].

1.3. Objectives

1.3.1. General objective

The major objective of this study was to improve the electrochemical performance of graphite anode by MnO₂ based nanocomposites in MFC systems for better operational applications. The work was focused on PANI encapsulation *via* MnO₂/NiO based composites to increase electron transfer kinetics, realize power, and treatment efficiency between the microbe and modified graphite anode in MFC.

1.3.2. Specific objectives

From the general objective the following specific objectives were listed:

- To synthesis MnO₂ NPs, MnO₂/NiO, MnO₂/PANI, and MnO₂/NiO/PANI nanocomposites using *Vernonia amygdalina* leaf extract.
- To characterize the optical, surface topography, thermal and, electrochemical, properties of the synthesized materials.
- To prepare different catalyst modified anode by drop-cast coating method and identify a suitable anode material using voltammetry and impedance spectroscopy techniques.
- To optimize and examine factors (*e.g.*, temperature and pH) that affects the performance of nanocomposite modified electrodes in MFC system.
- To observe the morphological characteristics of anaerobically cultured *E. coli* cells immobilization onto MnO₂ based nanocomposites.
- To evaluate electricity generation and biodegradation performances using doubled chamber MFC by using glucose and methyl red as a test sample in the presence of *E. coli* species.

1.4. Significance of the study

A day to day interest has been growing on MFCs to generate electricity *via* biodegradation of organic waste matters in contrast to conventional bioremediation based techniques. Therefore, from this study, the following unique advantages have been summarized.

- The finding of this study have a role to degrade wide range of organic waste matter in wastewater and simultaneously generate electricity using a reusable composite modified anode material.

- MFC has good effluent quality and less sludge production due to the better COD conversion efficiency.
- Unlike other bioremediation, anaerobic digestion, and other fermentation processes, in this study MFCs were operated at a mild conditions such as neutral pH, even at low wastewaters strength and ambient temperature.
- No need to waste energy on heating and no heat generation were formed during the operation rather the chemical energy stored in the organic waste matter were recovered in the form of power density.
- In most conventional bioremediation techniques, explosion on the reactor might happened and causes for accidents but this is not common in MFC.

So, this study would give a practical application and value added encouragement to Ethiopia by producing a renewable bioelectricity with simultaneous waste utilization.

1.5. Scope of the study

The study covered from bioremediations to bioelectricity generations and focuses on synthesis, characterizations of synthesized MnO_2 NPs based nanocomposites, and composite modified anode electrochemical catalytic activities to achieve efficient water treatment and energy production from organic waste resources. Conventional bioremediations from anaerobic digestion to phytoremediation methods are economically safe and energy-efficient biological process to produce energy and to treat wastewater from a variety of organic waste streams. However MFCs potentially have a much wider scope and possibility of efficient energy generation than anaerobic digestion in the future. But poor understanding of interactions between materials and electroactive microbes make in depth investigations of MFC essential to try to achieve efficient energy production with simultaneous waste remediation from waste resources. In this dissertation, using nanocomposite as a catalyst is useful to enhance electron transfer rate between microorganism and anode electrode that possibly lead to a wide range organic wastes to produce a sustainable energy and to exploit waste resources efficiently.

2. LITERATURE REVIEW

2.1. Transition metal oxide NMs

Families of first row based TMOs are excellent candidates for a promising research interest in batteries, capacitors, and fuel cell applications due to their reversible structural stability, rich structural variety, high catalytic activity, non-toxicity and environmental friendliness with good electronic conductivity [52]. They have a vast bioelectrocatalytic application in MFC performance. But their minimum size, smaller than cells and cellular organelles, allows them to penetrate these basic biological structures, disrupting their normal functions. Examples of toxic effects include tissue inflammation, and altered cellular redox balance toward oxidation, causing abnormal function or cell death [53]. An important mechanism of nanotoxicity is the generation of reactive oxygen species, resulting in the subsequent formation of oxidative stress in tissues [54]. In MFC at the anode site the possibility of generating reactive oxygen is diminished due to anaerobic conditions. But to control bacterial cell damage from such NMs, coating them with biocompatible redox polymer by forming an electroactive biofilm have been the best strategy to control the toxicity effects of NMs [55].

2.1.1. Manganese oxide NPs

Among TMOs NMs, MnO_2 is one of the general classes of manganese oxides (MnO , MnO_2 , Mn_2O_3 , and Mn_3O_4) that exhibit rich in structural and morphological properties, due to its unique crystalline structure arrangement and presence of rich valences of manganese (Mn). It often contains foreign cations and structural vacancies [56], with attracted enormous interest due to its high stability, availability, low cost, and effective catalytic properties in an electrochemical reaction [57]. It has α -, β -, γ -, δ -, ϵ - and λ -types of structural forms, when the basic structural unit ($[\text{MnO}_6]$ octahedron) is linked in different ways. Based on this links, MnO_2 can be divided into three categories: the chain-like tunnel structure such as α -, β -, and γ -types, the sheet or layered structure such as δ - MnO_2 , and the 3D structure such as λ -type. Thus its properties is clearly affected by their phases and morphologies; moreover, the operating properties of MFC and waste remediation techniques are strongly depends on the phase of MnO_2 [58]. But its poor electrical conductivity and instability in high proton conducting media is its major drawbacks for its effective utilization limits its long range applications [59]. In terms of energy storage conversion mechanism, MnO_2 presents with an extraordinary theoretical specific capacitance of 1370 Fg^{-1} , which is

associated with poor electrical properties. To avoid this limitation, combination with conducting polymers to form hybrid composites offers an interesting alternative directed to improved electrical properties [60].

Thus, the significant interest to synthesis and obtain a well dispersed, uniform, and an ultrafine MnO_2 NPs have been a current strategy for wide range of applications [61]. Previous work has been done to improve MnO_2 NPs performance through several chemical methods, such as immersion [62], hydrothermal [63], electrochemical [64], precipitation [65], and quick chemical precipitation technique at different temperature [66]. In chemical method, chemicals directly involve during synthesis which can impart toxicity to the prepared NPs. To improve these, studies have been found to prefer less toxic chemicals as reducing agents, but their preparation strategy still took place in either acidic or basic environment for MnO_2 NPs synthesis without the use of any surfactant. In addition to its toxicity, the prepared NPs might aggregate on long time storage and might impart toxicity at the bare surface of the reactive electrode. So, to avoid these problems following greener route of NPs synthesis is preferred. Hence, biosynthesis using plant leaf extract which is adopted from green chemistry approach offers a minimum toxic products and by-products [67]. Therefore, in this dissertation biosynthesizing of optimized MnO_2 NPs was prepared using aqueous *Vernonia amygdalina* leaf extract as a reducing and/or stabilizing agents.

2.1.2. Nickel oxide NPs

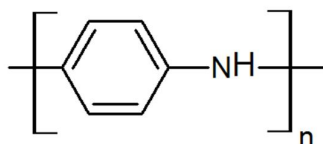
Nickel oxide (NiO) is emerging and important TMOs with cubic lattice structure. Like, MnO_2 , NiO has been studied as a low cost, environmental friendly, and favorable alternative electrocatalyst in batteries, super-capacitors, and MFC due to having high theoretical capacitances [68]. Its minimal environmental impacts and high capacitance could be good option for anode materials in MFC. Since NiO alone has also low electrical conductivity and this restrict MFC performance. To overcome this problem, it is modified with other new materials or conductive polymer [39]. So, it exhibited a good anode modifier with excellent durability due to change in quantum size and surface effect [69].

NiO based composite has been found to exhibit a good electrocatalytic activity towards the bacteria metabolites due to its excellent capability in transferring electrons from bacteria to anode in MFC. Due to its multiple desirable effects, NiO was used as electron mediators to accelerate the electron transfer between the bacterial cells and conventional carbon electrode when combined with carbon nanotube and PANI due to its synergetic effects on MFC anode

materials [68]. So, in this research incorporation of NiO with MnO₂ and PANI by forming good functional nanocomposites was prepared and played an important role in fast electron transfer mechanism between *E. coli* and anode surface without reducing their viability in MFC.

2.2. Conducting polymer NMs

The nanostructured conducting polymers materials permit rapid electron transfer kinetics for analytes, leading to fast response/recovery time even at room temperature [70]. The most notable property of conducting polymers is their inherent electrical conductivity, which is closely connected to the charge transfer rate and electrochemical redox efficiency. For example, PANI and its derivative become the leading polymeric materials in various fields of applications due to its low oxidation potential and good biocompatibility. Its chemical structures are given in Scheme 2. It has the ability to share its strength for other materials due to having low-temperature synthesis and processing, large-area manufacture, flexibility, and cost effectiveness. PANI, PPy, and poly(3,4-ethylenedioxythiophene) have been synthesized in the form of powder and film using chemical and electrochemical polymerization methods, respectively [71].



Scheme 2. Chemical structure of PANI.

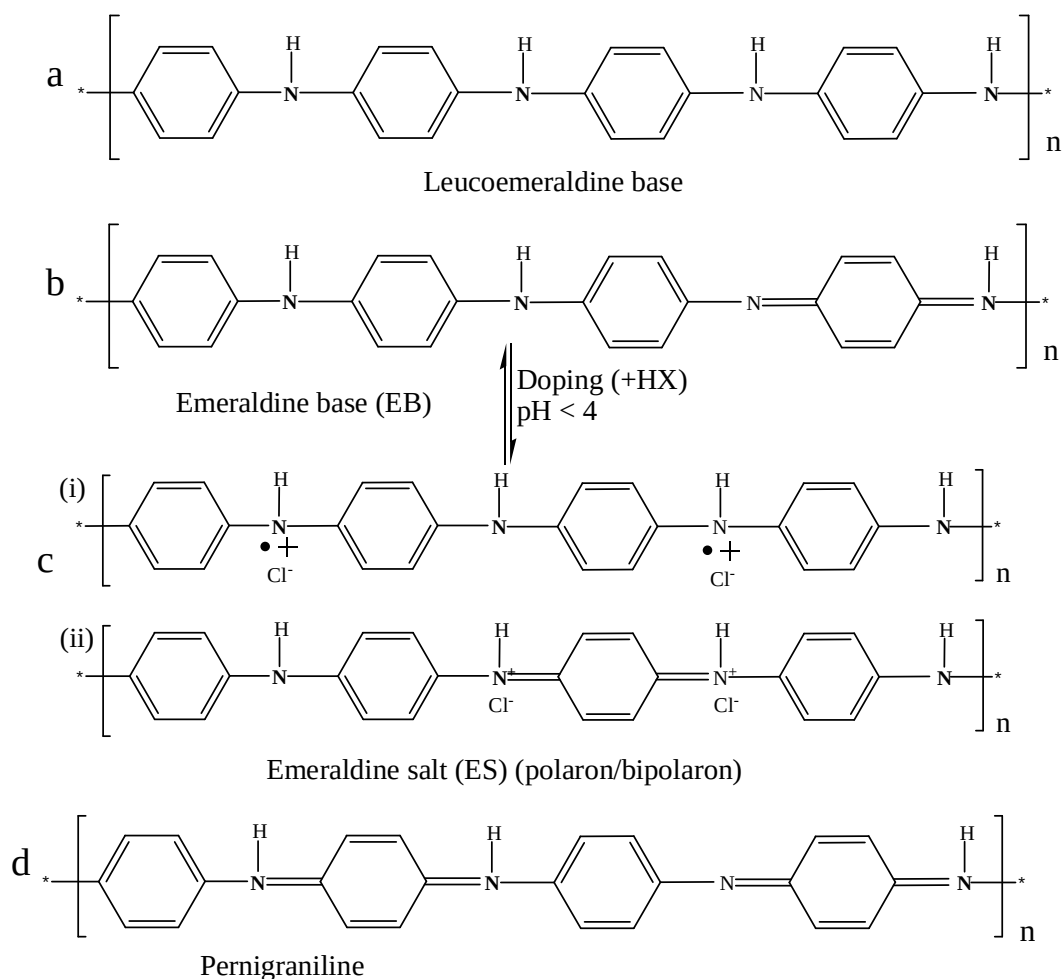
PANI is one of the members of the intrinsically conducting polymers that undergo a reversible acid/base doping/de-doping chemical property [72]. The presence of nitrogen heteroatom in between phenyl rings across its backbone chain, presence of delocalized electronic states, and charge transfer mechanism results for the formation of polarons and bipolarons in the energy band led to the possibility of different types of charge conduction. PANI can act as either a direct electrode or indirect supporting material. To date, PANI has proven to be an ideal supporting matrix for the immobilization of metallic NPs, resulting in electrode materials with superior performance and dispersive ability to prevent agglomeration of metallic catalysts [73].

From the CV report, PANI has been existed in three main oxidation states. These are leucoemeraldine base (LB or LE, colorless or white in color, 100% C-NH-C amine bonds, *i.e.* fully reduced state), emeraldine base (EB, blue in color, 50% C-NH-C amine bonds and

50% C=N-C imine bonds, insulating) which exists in a semi-oxidized state or emeraldine salt (ES, green in color, conducting) after doping giving rise to polaron and bipolaron structures) with an acidic doping agent, and pernigraniline base (PB or PG, purple in color, 100% C=N-C imine bonds, fully oxidized state) as shown in Scheme 3 and n stands for number of polymerization degree [73]. Insulating emeraldine base consists of two amino nitrogen atoms followed by two imino nitrogen atoms and is blue in color (Scheme 3b). The acidic doping agents allows the protonation of the imine nitrogen atoms of the emeraldine base to form the emeraldine salt (Scheme 3c). Doping agent, the regular distribution of the counter-ions of the acidic doping agent contributes to increase charge carriers mobility. So, doping process allows PANI to change from an insulating state to conductive state with a crystalline (ordered) region responsible for its conductivity, dispersed in an amorphous (disordered) region [73].

The most common types of oxidizing agents are coming from APS, $K_2Cr_2O_7$, H_2O_2 , $KMnO_4$, and KI. All this oxidants allow the elimination of a proton from the aniline monomer and the initiation of polymerization by redox processes involving the repetition of its monomeric units. The amount of oxidizing agent during PANI polymerization influences the amount of radical cations and thus the polymerization yield; however, an excess of an oxidizing agent can lead to excessive oxidation of PANI, while a deficit may be insufficient to obtain the required polymer. Thus, the control of oxidizing agent per aniline monomer ratio has a critical importance [73].

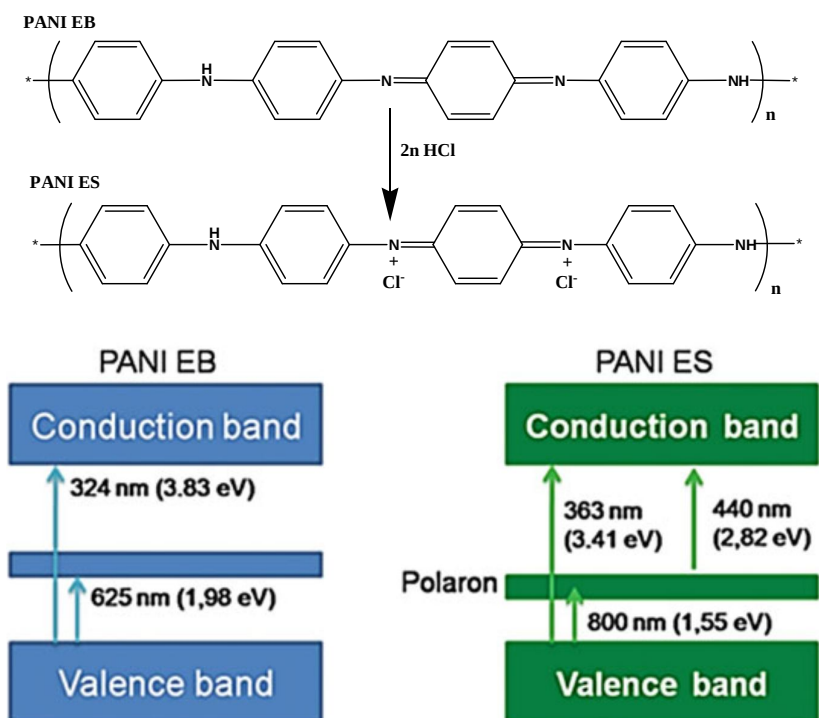
It is noted that, the emeraldine form of PANI is often referred to as EB that is neutral and is regarded as the most useful form of PANI due to its high stability at room temperature and the fact that, upon doping with acid, the resulting emeraldine salt form of PANI is high electrical conductors while leucoemeraldine and pernigraniline are poor conductors, even when doped with an acid. The most common green protonated emeraldine is produced by oxidative polymerization of aniline in aqueous acids and has conductivity on a semiconductor level of the order of 10^0 S cm^{-1} , many orders of magnitude higher than that of common polymers ($<10^{-9} \text{ S cm}^{-1}$) but lower than that of typical metals ($>10^4 \text{ S cm}^{-1}$) due to the presence of cation radicals in its structure. The positive charge on aniline units is balanced by negatively charged counterions, typically chloride or sulfate anions [74].



Scheme 3. Chemical structure of (a) leucoemeraldine, (b and c) emeraldine base or emeraldine salt, (d) pernigraniline.

The overall PANI energy diagrams like metals and TMOs NMs in terms of conduction and valence band and its structure before (emeraldine base or EB) and after (emeraldine salt or ES) doping is stated in Scheme 4 [75]. For example, consider PANI synthesis doped by HCl. It is important that the chemical synthesis parameters based on the oxidizing agent, acid doping agent and counter anions have a great influence on the oxidation state and the doping level of PANI, resulting in various final morphologies and properties.

For anodic electrode modification in MFC, PANI can reduce extracellular electronic transmission resistance between electrodes and microorganisms, increase specific surface area of electrodes, enhance microbial adhesion to the surface, and enhance bioelectricity generation and bioremediations in MFC device system [39]. Consequently, it has the capacity to improve the bioelectrocatalytic performance in MFC [25].



Scheme 4. Energy diagrams and PANI structure before (emeraldine base or EB) and after (emeraldine salt or ES) doping.

2.3. Composite NMs

Nanocomposites are a new generation of novel materials, which are formed by mixing one or more dissimilar materials at the nanoscale in order to control and develop new and improved structures with various architectures design as shown in Figure 3 [76].

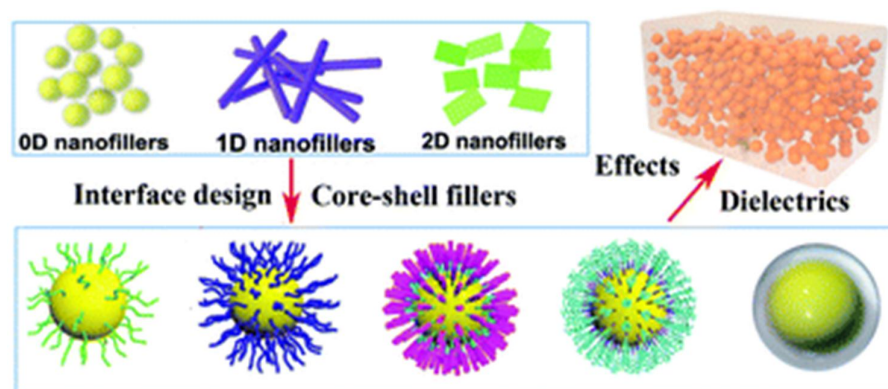


Figure 3. Dimensions and interface design architectures in nanocomposites [76].

The properties of nanocomposites depend not only upon the individual components used, but also upon the morphology and interface characteristics. Enhanced properties such as

improved friction and abrasion resistance, fouling resistance, super hydrophobicity, super hydrophilicity, thermal energy transport, electronic and ionic transport, and liquid transport displayed by nanocomposites compared to individual materials make them attractive for a wide range of engineering applications [77]. The properties of nanocomposites rely on a range of variables; particularly the matrix reinforcing materials, which can exhibit nanoscale dimensions, loading (percentage by volume, percentage by weight), degree of dispersion, size, shape, and orientation of the nanoscale second phase and interactions between the matrix and the second phase. So, the nanosized materials can be produced from metals, metallic oxide, and polymers to modify their physical, chemical, and mechanical properties of the composite materials [78].

2.3.1. Metal oxide-metal oxide composites

Metal oxides play a very important role in many areas of chemistry, physics and materials science. The metal elements are able to form a large diversity of oxide compounds with a number of structural and geometrical characters, such as metallic, semiconductor or insulator. Noble metal NMs have received much attention in the past decade because of their unique chemical and physical properties, and they have been widely applied in pollutant degradation, waste minimization, energy conversion, and so forth. However, for most technologically important chemical processes, noble metal catalysts spontaneously aggregate and grow to reduce surface energy, which limits the lifetime of NMs and efficiency. To satisfy the urgent demand for high performance catalysts with the characteristics of excellent activity, good selectivity and remarkable stability, catalysis, especially heterogeneous catalysis, has been undergoing comprehensive studies and innovative advances accompanied by great promise in nanoscience and nanotechnology over the past decade [79].

Making a composites is not only provides a high surface area to stabilize small NPs under long-term catalysis but also renders hybrid junctions with rich redox reactions on the two-phase interface [79]. For long term energy conversion and toxic chemical removal, porosity characteristics in the mixed metal oxide based nanostructured material is a big criteria [80]. In the nanotechnology prospective, materials with organic and/or inorganic frameworks having pore size of nano dimension (100 nm or less) in their structure are termed as nanoporous materials. The presence of pores (holes) in a material can introduce many important properties which the corresponding bulk material does not possess. They can be

subdivided into three main categories: microporous (pore diameter < 2 nm), mesoporous (pore diameter 2-50 nm) and macroporous (pore diameter > 50 nm) [80].

Porous metal oxide composites are the composites of two or more oxide materials having porosity in the framework. These composites are derived from mixed oxide, oxide composite, and metal-metal oxide type (M1/M2O). In oxide composite formation of two distinct phases (*i.e.*, M1O/M2O) while in the mixed oxide type, the formation of a single phase from the two different metal species (*i.e.*, M1M2O). For clear understanding see Figure 4 [80], where M1 and M2 represents metals.

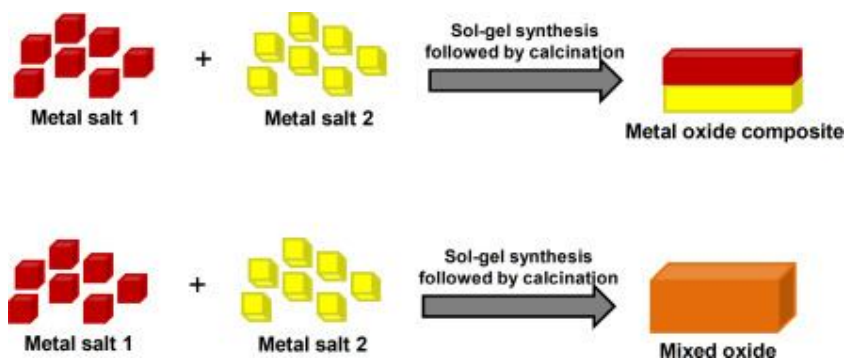


Figure 4. A picture to clarify the difference between metal oxide composites and mixed oxide [80].

2.3.2. Metal oxide-conducting polymer composite

The electrochemical catalytic performance of composites can also be improved by combining TMOs and conducting polymers. Due to their fast/reversible redox reactions and high specific energies, both TMOs and conducting polymers can synergistically offer superior charge storing capacity (capacitance) due to the excellent physico-chemical properties and stability of conducting polymers in the electrochemical systems as shown in Table 1 [81,82]. The homogeneous distribution of metal oxides on conducting polymer matrix improved electrical conductivity, stability, capacitance, surface area increment, and aggregation reduction of metal oxides due to polymer capping agent activity. This modification helps to shorten electron transfer pathways followed by providing more active surface sites for energy conversion and charge storage applications [83]. Individual materials still suffer from some limitations including the poor conductivity of TMOs, which contributes to a low energy conversion and storage capacity and causes the structural destruction of the TMOs and conducting polymer materials during the doping-dedoping process, thus leading to a decrease in stability performance.

Table 1. Theoretical and experimental specific capacitance of conducting polymers [82].

Conducting polymer	MW (g mol ⁻¹)	Dopant level	Potential range (V)	Theoretical specific capacitance (F g ⁻¹)	Experimental specific capacitance (F g ⁻¹)
PANI	93.00	0.50	0.7	750	240
PPy	67.00	0.33	0.8	620	530
PTh	84.00	0.33	0.8	485	-
PEDOT	142.00	0.33	1.2	210	92

PANI = Polyaniline; PEDOT = Poly[3,4-ethylenedioxythiophene]; PPy = Polypyrrole; PTh = Polythiophene; MW = Molecular Weight.

The very poor electronic conductor of conducting polymers in the neutral state is going to be the question for improving its properties. These problems limit the electron transfer rate capability of an electrode. Thus, a number of metal oxide particles have been encapsulated by conductive polymer matrix to form nanocomposites. So, TMOs are promising candidates for electrocatalytic applications because of possible pseudocapacitance over a wide range of potential. However, they generally suffer from poor electrical conductivity. Hence, the formation of conducting polymer-metal oxide composite for electrode modifications can achieve better electrical conductivity and electrochemical accessibility for fast surface redox reaction sites [84].

2.4. NMs preparation methods

2.4.1. Metal oxide NPs

Top-down (physical), bottom-up (chemical), and bio-inspired (green or biological) are the common method to synthesis metal oxide NMs. The high energy consumption and the need for expensive equipment and facilities found in physical methods are the main drawbacks to seek on bottom-up approach. The bottom-up approach offers more control over the size, shape, and composition of nanostructures and do not require expensive equipment and is more suitable for large-scale production. But consuming more time during synthesis are its limitation [85]. To reduce these problems, emphasis is given to the bio-inspired method and briefly discussed in the following section.

Green method (bio-inspired methods): In general, the chemical-based bottom-up approaches are preferred for the synthesis of metal oxide nanostructures, owing to low growth temperature, cost-effectiveness, and better potential for mass production. However, many chemical bottom-up methods typically employ toxic chemical compounds as surfactants or capping agents which are environmentally unfriendly and difficult to degrade. To reduce this, efforts have been chosen green chemistry approach for nanostructured

fabrication [86]. So, the green synthesis process is a unique method that leads to saving on energy, time, and cost. Natural biological products from various plant sources have been attracting great interest as natural reducing and stabilizing agents for the green and eco-friendly synthesis of metal oxide nanostructures. Leaves, flower, roots, and fruit peels or vegetables contain biologically active compounds known as phytochemicals are utilized to synthesis metal oxides nanocatalysts. To obtain such compounds, an important steps are illustrated in Figure 5 [86].

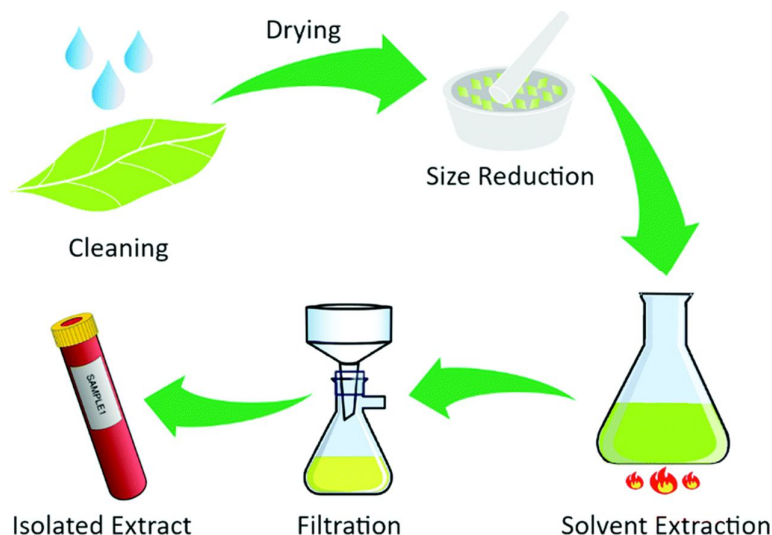


Figure 5. Schematic diagram showing the general steps for obtaining phytochemicals from plants [86].

Reducing Agent: Reducing agent is used to reduce metal ion species. The reduction process strongly depends on reduction potential of the involved species. Based on this various metal NPs are produced due to having higher positive reduction potential than plant extracts. All phytochemicals from plant extracts can help to reduce and stabilize metal ions followed by preventing agglomeration. The oxidation of metal to metal oxide nanostructures are achieved by calcination under air or oxygen-rich atmosphere [86].

Capping Agent: Capping agent is a compound which suppresses particle growth by passivating the surface of particles followed by reducing the surface energy of particles so that aggregation can be avoided. Its role is acting as a surfactant to control the NPs crystal growth. There is a very strong relationship between the particle size and the size of the surfactant or capping agent. The larger the size of the capping agent exactly describes the

smaller the size of the obtained NPs. So, from the plant extracts flavonoids and phenolic acids act as a reducing and capping agents simultaneously [87].

In detail, plant secondary metabolites contained from polyphenols, phenolic acids, sugars, terpenoids, proteins and alkaloids etc., which are very crucial to reduce metal ions from their precursor to their corresponding NPs. For example, carbonyl and hydroxyl functional group situated at C₃ and C₅ position of flavonoid (a polyphenolic compound) can effectively form a chelate with several metal ions [88]. For example, low molecular weight organic acids, such as oxalate found in *Rumex acetosa* extract was previously used to reduce iron (III) to iron (II), leading to the formation of Fe₃O₄. Oxalate ions can react with Fe³⁺ to generate CO₃²⁻ and Fe²⁺. The extract was added to the iron (III) chloride solution and the reducing process as well as the formation of Fe₃O₄ indicated color change from yellow to intense brown. During the process, oxalate ions could bind with iron oxide NPs surface by electrostatic interactions. Therefore, oxalate ion prevents NPs aggregation.

Bitter Leaf (*Vernonia amygdalina*) is derived from the leaves of a small ever-green shrub found in tropical Africa and Asia that belongs to Asteraceae and genus *Vernonia* family and used as a common traditional medicine in Ethiopia [89]. At least 13 other new compounds have been found in these leaves [90]. According to Alara et al. [91], ethanolic extract of *V. amygdalina* leaf contains flavonoids, alkaloids, steroids, terpenoids, glycosides, tannins, phenols, and the absence of anthraquinones. The presence of these phytochemicals and other secondary active molecules, such as resins, proteins, and carbohydrates in their leaves are responsible for anti-oxidants bioactivities [91]. Such bioactivities help to synthesis NPs having lower dimensional structure (<100 nm) (e.g., Ag₂O) [92]. In addition, those biomolecules helps for nucleation and growth mechanism in metal oxide based NPs preparation [93].

Qualitative and quantitative phytochemical classification evidenced that reduction of enol from Flavonoid to keto group might play a great key role in metal ions reduction. In a similar fashion, the deprotonation of OH-functional group from eugenol which is a class of terpenoids have the ability to reduce metal ions. So that as a conclusion, plant extracts are thus a strong and effective reducing agents in the active metallic based nanocatalyst synthesis [88]. From such advantage, in our studies an efficient α -MnO₂ and its bimetallic oxide nanocatalysts were synthesized using *V. amygdalina* plant extract in the aqueous environment.

2.4.2. Conducting polymer synthesis

Conducting polymers preparation highly depend up on their electrical conductivity strength. Due to their mobile charge carriers introduced into the π -conjugated system that is formed by the continuous overlap of extended and delocalized p-orbitals along the polymer chain's backbone. However, conducting polymers without doping generally exhibit very low conductivity at room temperature. According to the band model, the conductivity of those polymers are ranges from $10^{-10} \text{ S cm}^{-1}$ (an insulator) to about $10^{-5} \text{ S cm}^{-1}$ (a semiconductor) to greater than 10^4 S cm^{-1} (a metal or conductor), depending on the doping level.

Band model: A band model explains why some materials conduct electrical charge, while some do not, as shown in Figure 6. According to this model, the overlapping of individual molecular electronic states can produce electronic bands. The valence electrons overlap to form a valence band, while electrons in the conduction band have higher in energies, which are sufficient to allow electrons to move freely within the materials. The energy difference between these two bands is called band gap energy (E_g). In metal conductors, the valence band and the conduction band overlap; thus, electrons can move freely in a background of positive charge formed by the ion cores. The band gap of insulators is generally very high, resulting in a low conductivity at room temperature. The electrons then become mobile across π -conjugation, and the material is termed a semiconductor. The overlapping of the π -bands is the valence band, and the π^* -band is the conduction band in the conducting polymers. If the band gap is removed by further extending the π -conjugation, a conducting polymer can be as conductive as a metal [94].

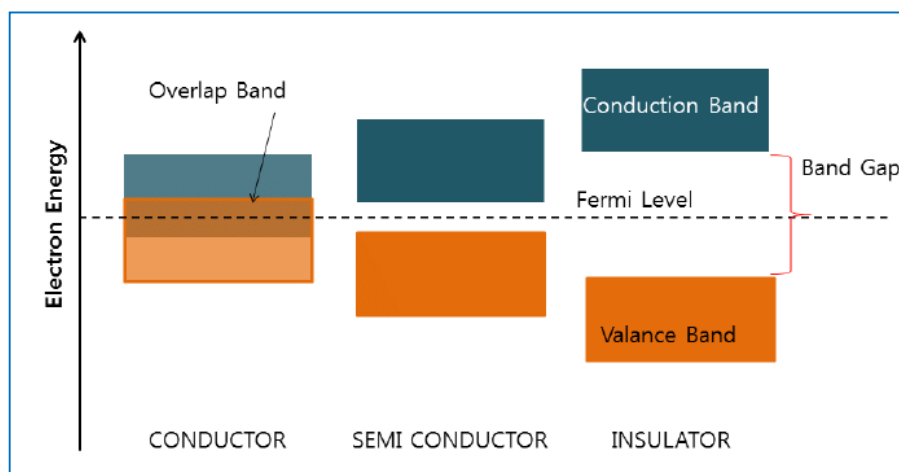
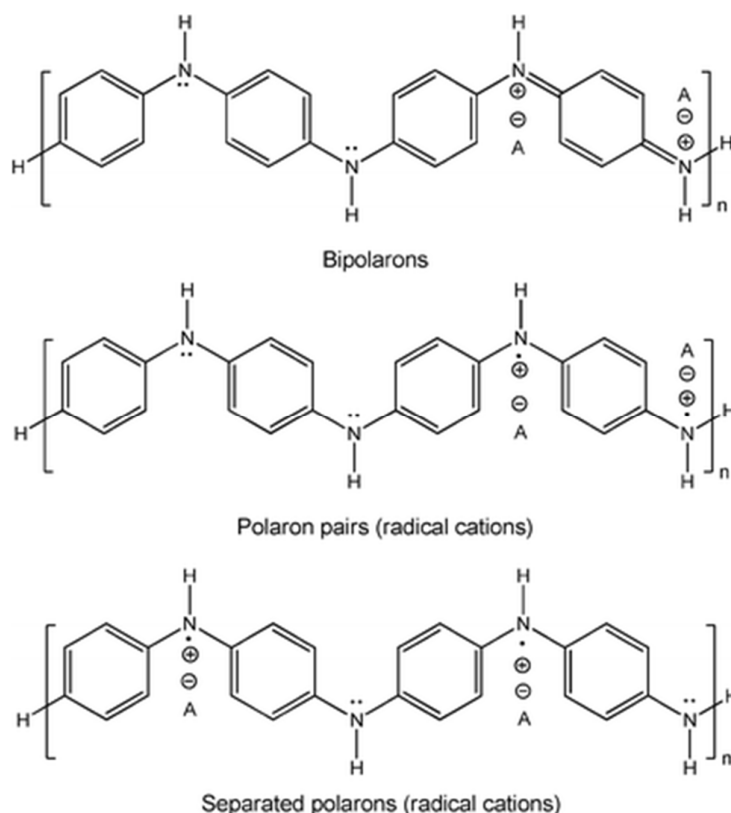


Figure 6. Schematic figure of band model [94].

Doping: This is the process of introducing chemical species to increase conductivity. Which may be either Lewis acid or inorganic acid such as, FeCl_3 , HCl , HNO_3 , H_2SO_4 , H_3PO_4 , etc. It has several advantages, such as charge transfer by either oxidation (p-type doping) or reduction (n-type doping). During doping, overall neutrality and the simultaneous control of chemical potential is also governed by the associated counter ion. p-type doping, achieved by chemical or electrochemical oxidation, is essentially the removal of electrons from the valence band, leading to the presence of positive charges on conducting polymers. Then, the released electrons could be added to conduction band, causing an n-type doping. p-type doping is generally more common than n-type because most n-type doped conducting polymers are not as stable in the air due to oxidation by O_2 [95].

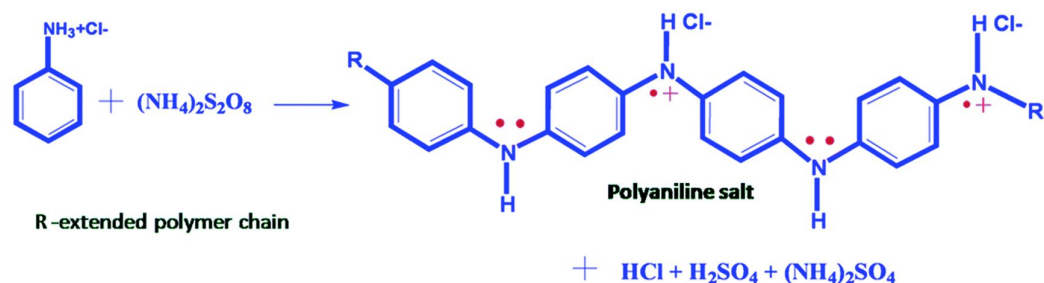
During doping charge carriers, such as polaron, bipolaron, and solitons could be formed by a redox reaction or protonation. Polaron is resulting from local distortion of conducting polymer structure, followed by removal of single electron. If another electron is further withdrawn from valence band, a bipolaron can be generated as shown in the Scheme 5 [96].



Scheme 5. Illustration of PANI repeating units for bipolaron, polaron, and separated polaron forms [96].

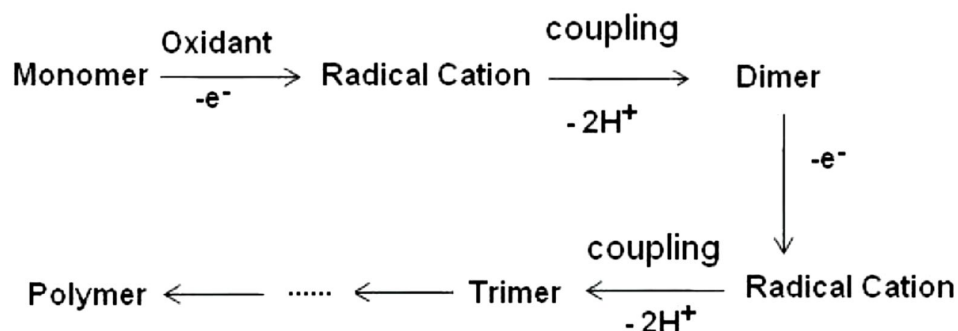
2.4.2.1. Chemical methods

Conducting polymers have been polymerized using Chemical or Electrochemical method. Chemically conducting polymers have been synthesized through the oxidation or reduction of monomers and polymerization of corresponding monomers. One of its advantages is the chance of mass production at a reasonable price. In this polymerization, a monomer, a dopant, and an oxidant are dissolved in a solution kept at a certain temperature (see Scheme 6) [97]. The monomer is first oxidized into a radical cation, which has several resonance forms of cations. The coupling of two radical cations results in a dimer. The dimer can then be oxidized into a dimer radical cation and continuation (propagation) of these reactions produces oligomers followed by polymers until termination of the chain as shown in Scheme 7 [98]. The mixture is then filtered, washed, and dried to yield pure conducting polymers.



Scheme 6. Chemical polymerization of aniline monomer by ammonium persulfate yielding PANI emeraldine salt [97].

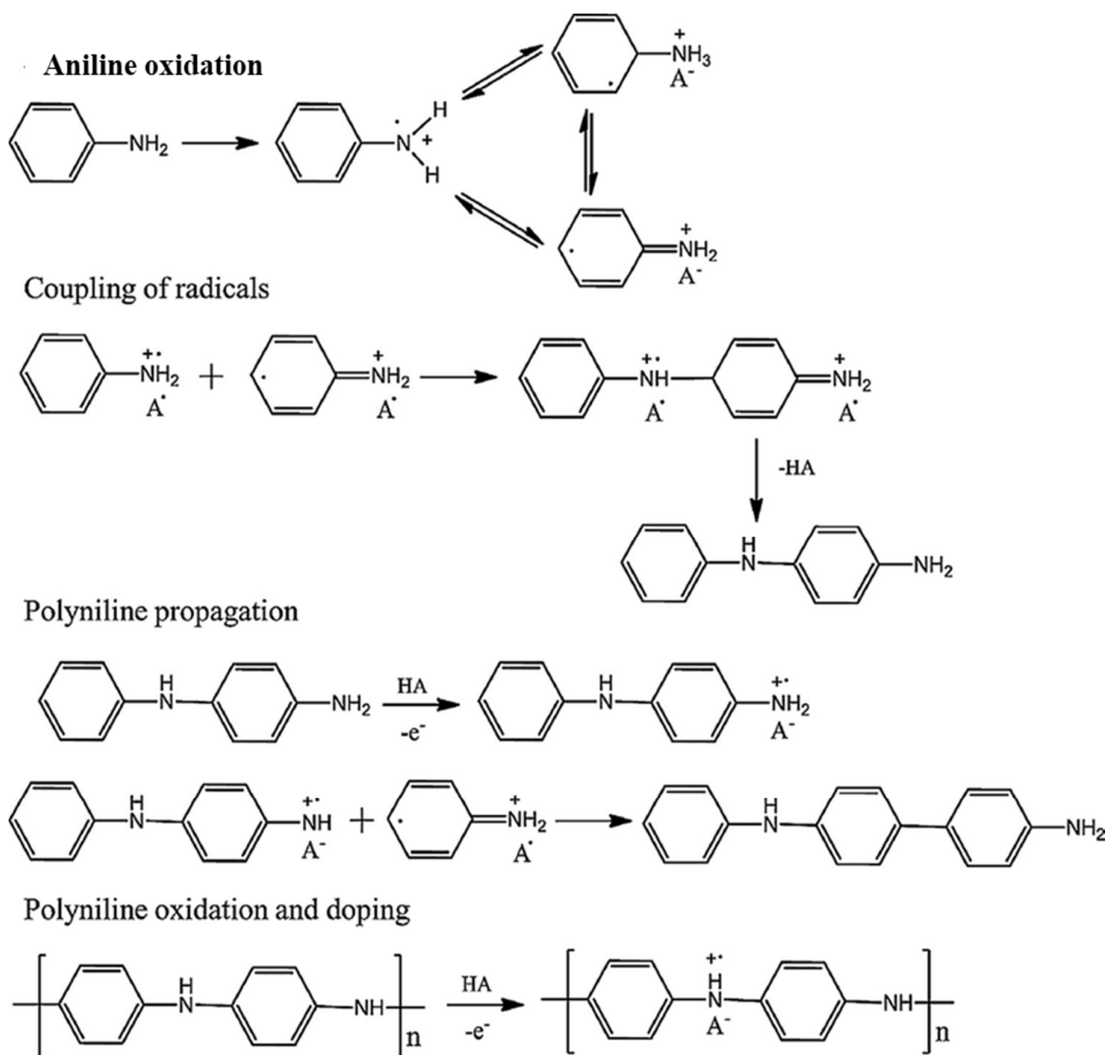
Successful polymerization to high molecular weight entails that oligomers and low molecular weight polymers be reactive and soluble sufficient to polymerize. High conjugation polymerization is stable.



Scheme 7. Polymerization scheme of a conducting polymer [98].

2.4.2.2. Electrochemical methods

Similar to chemical polymerization, the radical cation is generated at the initial step *via* an applied potential in three-electrode (working, reference, counter electrodes) or a two-electrode (working and counter electrodes). In a typical electrochemical route, a potential is applied across an electrolyte solution containing a monomer and a dopant. Electrochemical polymerization is convenient, since the polymer does not need to be isolated and purified. In both polymerization cases, the initial step is the formation of the radical cation, followed by coupling reaction of radical cations as illustrated in Scheme 8 [99].



Scheme 8. Electrochemical polymerization mechanism of PANI [99].

2.5. Characterization techniques

2.5.1. Ultraviolet-Visible (UV-Vis) spectroscopy

UV-Vis absorption spectroscopy is the technique by which properties of unknown samples are measured by interacting light with sample or also after reflection from the sample. The light is energetic that can excite electrons to higher energy levels [100]. Its principle follows the Beer Lambert law which states that absorption of light by the sample is directly proportional to the path length and concentration of the sample as given from the formula:

$$A = \log \left(\frac{I_0}{I} \right) = \epsilon cl \quad (1)$$

Where A is absorbance, I_0 is intensity of light incident upon sample cell, I is intensity of light leaving sample cell, C is molar concentration of sample, l is length of sample cell (cm), and ϵ is molar absorptivity.

When light falls on the sample each material absorbs a specific range of light and electron from the atom excites towards the higher state from the lower energy state. These two states are LUMO (Lowest unoccupied molecular orbit) and HOMO (Highest occupied molecular orbit). There are further classification of bonds and these bonds showed the specific behavior of the sample which is being analyzed under the light and their ways of transitions as shown in Figure 7 [101].

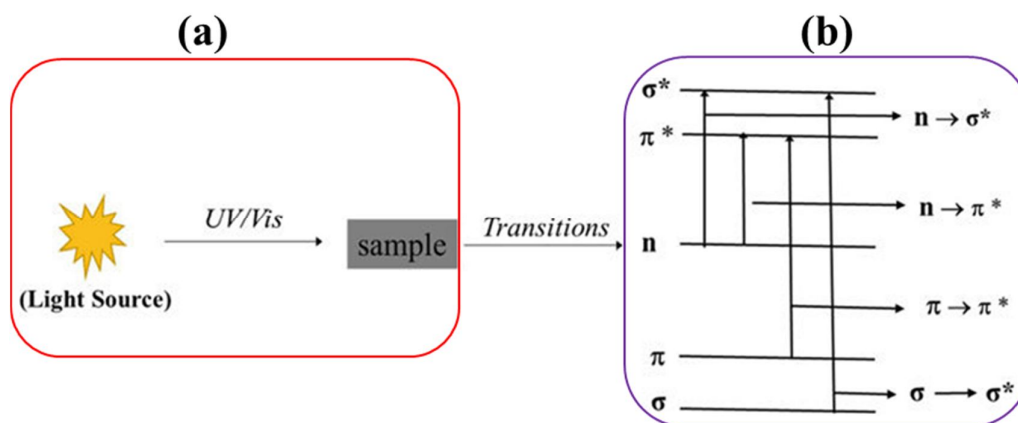


Figure 7. (a) Sample-light interaction scheme and (b) Electron transition diagram [101].

It has a directed application towards detection of chromophore functional group, determine the unknown compound, purity of the sample, and band gap calculations. In the energy band gap determination at a maximum wavelength, the following equation is applied.

$$E = \frac{hc}{\lambda} \quad (2)$$

Where E is energy band gap, h is planks constant, and c is speed of light λ is the maximum wave wavelength at maximum absorbance.

Generally, the electronic transitions, the transition band gap, and oxidation state of the metal ions present in the oxide frameworks can be found out from UV-Visible absorption measurements in the wavelength region from 200 to 800 nm. From the absorption, several peaks could be formed in terms of $L \rightarrow M$, $M \rightarrow L$ or $M \rightarrow M$ charge transfer transitions (L = ligand, M = metal) of the TMOs. In the spectra of A_mO_n/B_mO_n (where A and B are transition metals while m and n are oxidation states) composites indicate the charge transfer transition $O^{m-} 2p$ to $3d A^{n+}$, and to $3d B^{n+}$ charge transfer bands [80]. Example, several broad peaks in UV-Visible absorption spectra of $NiTiO_3/NiO$ composites indicate the charge transfer transition $O^{2-} 2p$ to $Ti^{4+} 3d$, Ni^{2+} to Ti^{4+} charge transfer bands.

2.5.2. Fourier transform infrared (FTIR) spectroscopy

FTIR measures the absorption of IR radiation made by each bond in the molecule and as a result gives spectrum which is commonly designated as % transmittance *versus* wavenumber (cm^{-1}). The IR region is at lower energy and higher wavelength than the UV-visible light and has higher energy or shorter wavelength than the microwave radiations. For the determination of functional groups in a molecule, it must be IR active (*i.e.*, molecule having a dipole moment). When the IR radiation interacts with the covalent bond of the materials having an electric dipole, the molecule absorbs energy, and the bond starts back and forth oscillation. Therefore, the oscillation which caused the change in the net dipole moment of the molecule should absorbed IR radiations [101].

The plot of measured infrared light intensity (absorbance or % transmittance) *versus* its property, such as energy range expressed in wavenumber (cm^{-1}), is called an infrared spectrum. The IR spectrum is recorded in absorbance mode, which measures the amount of light absorbed by a sample, and its intensity at y-axis is plotted in absorbance unit *versus* wavenumber across x-axis. On the other hand, the y-axis of an infrared spectrum can be also plotted in unit called percent transmittance (% T), which measures the percentage of light transmitted by the compound as follows:

$$\%T = 100 \times (I/I_o) \quad (3)$$

where % T is percent transmittance; I_0 is intensity in the background spectrum; and I is intensity in the sample spectrum.

2.5.3. X-ray Diffraction (XRD) Spectroscopy

Typically X-ray is an electromagnetic radiation having a wave length of $1\text{\AA}$ in between ultraviolet and gamma-rays. In materials science X-rays diffraction is known as a characterization technique capable of investigating the crystalline structures of the grown nanostructures. The interaction of X-ray beam with crystal resulted in a diffraction pattern that identifies the material and corresponding phase. The information obtained from the diffraction pattern includes size of the crystallite, symmetry of the unit cell, etc. Remember that the set of miller indices in each interparticle distance is unique for each and every material. It is important to point out that by manipulating different parameters like the geometry of incident rays and the orientation of the detector and crystal, one can obtain all the possible diffraction directions of the lattice. So, its quantitative parameters in terms of Bragg and Debye-scherer formulas are given in Equation 4 and 5, respectively [73]:

$$d_{(hkl)} = \frac{\lambda}{2 \sin \theta} \quad (4)$$

$$D = \frac{k\lambda}{\beta \cos \theta} \quad (5)$$

Where λ ($=1.54060\text{ \AA}$) is the X-ray wavelength, θ is the Bragg's angle, β (in radian) is the full width at half-maximum (FWHM) of the peak, k is a constant (≈ 0.9), D is the average crystallite size, $d_{(hkl)}$ is the inter-planar spacing of the crystallographic plane (hkl) [102].

2.5.4. Scanning electron microscopy (SEM)

SEM is one of the most important tools in order to study the overall appearance of the grown nanostructures and allows detailed investigation of the layers. It helps us in analyzing different parameters like quality, shape, diameter, thickness, length, and orientation of the grown nanostructures. An image is obtained by sweeping the solid surface in a *raster pattern* with a finely focused beam of electrons or suitable probe. A *raster pattern* is a scanning pattern in which the electron beam is first swept across the surface in a straight line (x direction), then returned to its starting position, and shifted downward (y direction) by a standard increment. The process is repeated until the desired area of the surface has been scanned. The beam of electrons passes through the electromagnetic lenses and strikes the surface of the sample. The bombardment of electrons does not cause any damage to the

samples. The detector collects the secondary/backscattered electrons ejected from the sample and converts them into a signal. Finally this signal is directed towards displaying screen. SEM has the ability to capture the images in the range of visible to few nanometers, while the magnification range is around $20\times$ to $30000\times$ along with a spatial resolution of 50 to 100 nm. The electron acceleration voltages are normally in the range of 5 to 20 kV. Further details can be found in reference [103].

2.5.5. Energy dispersive X-ray (EDX) spectroscopy

Energy Dispersive X-ray (EDX) is involved in different fields of study due to its high sensitivity in detecting different elemental composition. EDX coupled with SEM allows the amount of the main elements to be quantified (from 0.5 wt%) with $Z > 5$ [104]. EDX is able to identify and quantify all the elements in the periodic table except H, He and Li. One of the limitations of this technique is in the field of low Z elements detection due to the X-ray absorption phenomena [105].

The main concepts and operating principles of EDX is given as follows from nitrogen atom signal as a test example shown in Figure 8. At the atomic level, electron vacancies created from the incident electron beam are quickly filled by electrons of higher energy shells, releasing radiation of the difference in electron binding energies in the form of characteristic X-rays. These characteristic X-rays are energy-specific to the atom from which they were produced and thus reveal the elemental composition of a sample [106]. In brief, nitrogen has seven electrons arranged in two orbital shells: K and L. Electron binding energies of specified orbitals are shown (left). When an incident electron dislodges an electron from the K shell, inner-shell ionization occurs (middle). To fill the unstable vacancy, an electron from the L shell drops and the difference in binding energies is released as a characteristic X-ray. A K shell vacancy being filled from an L shell electron results in a $K\alpha$ line. This $K\alpha$ X-ray is used to fingerprint nitrogen in the collected spectra. The incident electron that created the vacancy has lost an amount of energy approximately equal to the binding energy of the ejected electron [106].

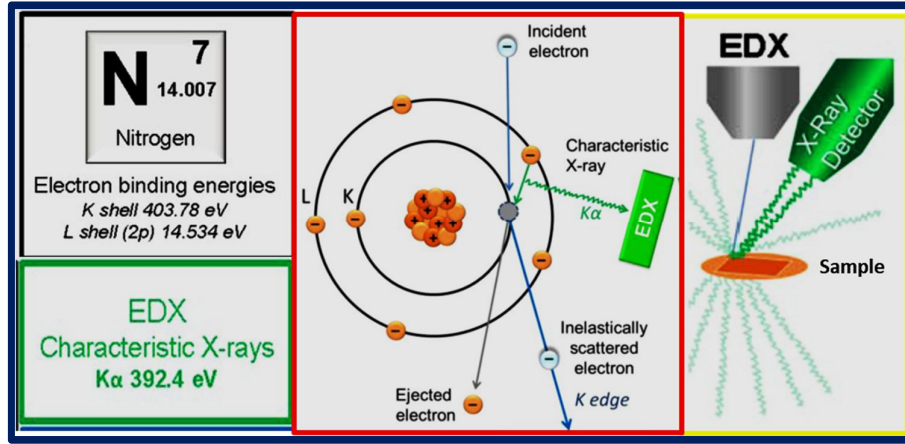


Figure 8. Working principle of EDX [106].

Therefore, EDX cannot detect trace elements (concentrations below 0.01 wt%) [107]. In conjugated conducting polymers, at low energy resolution, peak overlap may occur between the nitrogen and the conducting polymer carbon signal. To prevent this some researchers have used high energy resolution method [108].

2.5.6. Surface roughness

Several representative parameters should be selected to analyze the surface roughness. Among them, four parameters are common and selected for evaluating the surface characteristics: mean roughness (R_a), mean square roughness (R_q), surface skewness (R_{sk}), and kurtosis coefficient (R_{ku}). R_a is used to represent the mean value of the surface roughness of the sample. Their calculation equations are as follows [109]:

$$R_a = \frac{1}{N_x N_y} \sum_{i=1}^{N_x} \sum_{j=1}^{N_y} |z(i, j) - z_{mean}| \quad (6)$$

$$z_{mean} = \frac{1}{N_x N_y} \sum_{i=1}^{N_x} \sum_{j=1}^{N_y} z(i, j) \quad (7)$$

where the N_x and N_y are the number of scanning points on the x-axis and y-axis of the SEM image, respectively, $Z(i, j)$ is the height of the (i, j) measuring point, and Z_{mean} is the mean height of all measuring points from the SEM image.

The mean square roughness is used to represent the degree of change in the surface roughness of the sample. The calculation equation is:

$$R_q = \sqrt{\frac{1}{N_x N_y} \sum_{i=1}^{N_x} \sum_{j=1}^{N_y} (z(i, j) - z_{mean})^2} \quad (8)$$

The surface skewness is used to represent the symmetry of the surface height distribution of the sample [109]. If the value were zero, the surface height distribution would be the normal distribution whose symmetry axis was the mean value. A positive value means that the distribution is biased to the right side, indicating that there is more area where the sample surface height is below the mean value; a negative value means that the distribution is biased to the left side, indicating that there is more area where the sample surface height is above the mean value as shown in Figure 9a [109].

$$R_{sk} = \frac{\frac{1}{N_x N_y} \sum_{i=1}^{N_x} \sum_{j=1}^{N_y} (z(i,j) - z_{mean})^3}{R_q^3} \quad (9)$$

The kurtosis coefficient is used to represent the waveform characteristics of the surface height distribution of the sample. If the value were zero, the surface height distribution would be the normal distribution. A positive value means that the waveform is peaked, indicating that the surface height of the sample is concentrated at the mean value; a negative value means that the waveform is flat, thus indicating that the surface height of the sample is distributed (Figure 9b), and the equation is as follows [109]:

$$R_{ku} = \frac{\frac{1}{N_x N_y} \sum_{i=1}^{N_x} \sum_{j=1}^{N_y} (z(i,j) - z_{mean})^4}{R_q^4} - 3 \quad (10)$$

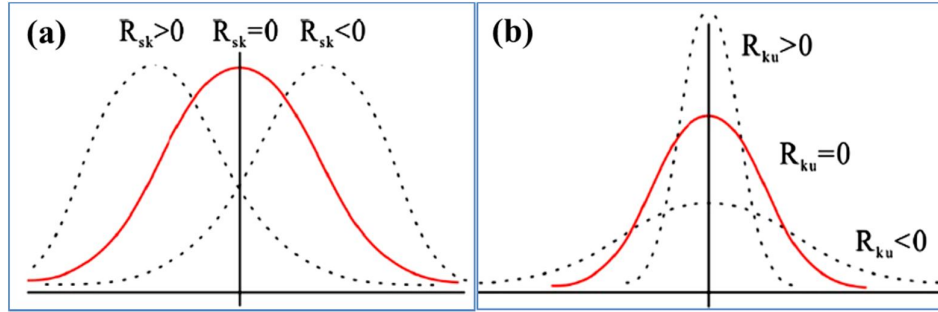


Figure 9. (a) Surface skewness (R_{sk}) and (b) Kurtosis coefficient (R_{ku}) shapes.

Quantitative evaluation of pores: The pore must be distinguished and marked before quantitative evaluation. The grains module of the Gwyddion software was used to mark and quantify particles in SEM images, and the invert height function was used to invert the height value of the marked pores. Segmentation method, threshold method, and watershed method can all be used to mark pores distribution. Among, threshold and watershed methods are the most commonly used. As for revealing complicated structures, the choices of threshold method decrease, while watershed methods are more widely used. The watershed

method is based on the principle that water flows to a region with a local minimum which represents a plane pore. The specific process is: (i) the water drops to each point on the surface of the sample; and (ii) the water flows to the region of the local minimum. Then the pores are identified with the pore size, plane porosity, pore surface area, and pore volume determined by the volume of water converging to the local minimum region [109].

2.5.7. Design of experiments

Traditional ways of NMs optimization using one factor at a time (OFAT) is changed while keeping all other variables constant. OFAT have the disadvantage of being time consuming, requiring more effort and materials, it cannot assess factor interactions, and covers a small fraction of the total feasible factor space during multiple experimental effect evaluation. To solve these limitations, design of experiments (DoE) is evolved. It is the most important aspect of response surface methodology (RSM) based on the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response [110]. It uses a number of designed strategies, such as Box-Behnken, central composite, factorials, and optimal coordinate (either I or D) exchange designs to understand the topography of the response surface including the local maximum, local minimum, and ridge lines and find the region where the most appropriate response occurs [111].

The advantages offered by RSM can be summarized as determining the interaction between independent variables, modeling the system mathematically, and saving time and cost by reducing the number of trials [111]. The general accepted strategy for DoE aimed at rapid, yet comprehensive, and process improvement. It begins with a (i) “Discovery” phase that calls for brainstorming and pilot studies that include range-finding trials. Many experimenters jump too quickly into a test matrix and end up wasting time on the wrong factors with ranges that are either too narrow, thus generating nothing significant, or too wide-going beyond operational limits. Then they must back up and start again by attempting to re-discover a proper starting point for their process and product development. The (ii) “Breakthrough” designs which vary all factors simultaneously *via* cleverly-devised matrices that compute effects with maximal power for predictive modeling. In fact, for a given level of statistical power, modern DoE methods require far fewer experimental runs than the OFAT approach.

Designs listed under the (iii) “Optimization” sections are known as response RSM, the basis of which is polynomial models fit by least-square regression and confirmed statistically *via* analysis of variance (ANOVA). By deriving empirical, but functionally useful, relationships-typically second-order-between process responses and the critical input variables, RSM facilitates discovery of ‘sweet spots’ where all requirements can be achieved at lowest cost. Armed with these polynomial equations, specialized software can apply numerical search algorithms that find the most desirable conditions. Finally, this recommendation must be validated *via* confirmatory tests as detailed in the final stage of the strategy for experimentation. Model accuracy from the evaluation of empirical model prediction, when the percent relative error between the predicted and validation output is falls as if the relative error <10% is excellent, 10% < relative error <20% is good, 20% < relative error <30% is fair, and >30% is poor [112]. This indicator is often used in statistics for estimating the performance of the models.

2.5.8. Differential scanning calorimetry (DSC)

Calorimetry is the general term for the measurement of thermodynamic changes in material due to chemical reactions, phase transitions, and other physical changes. It measures how physical properties of a sample change, along with temperature against time. Thermodynamic data (heat capacity, enthalpy and entropy) and kinetic data (rate of reactions, activation energies) can be measured with DSC. The difference in heat flows between the sample (T_s) and a reference material (T_R) are monitored and recorded as a function of temperature [113]. As a material undergoes a phase transition, more or less heat will be needed to maintain the sample temperature, depending on whether the transition is endothermic or exothermic. The difference in energy needed to match the sample temperature to the reference is the amount of excess heat released or absorbed as a result of sample transitions. In the most basic form, the information from DSC can be described by Equation [114]:

$$\frac{dH}{dt} = mC_p \frac{dT}{dt} \quad (11)$$

Where dH/dt is the DSC heat flow signal, m is the sample mass, C_p is the sample heat capacity, and dT/dt is the heating rate. Physical changes in the material, such as melting, crystallizing, and transitioning through glass transitions, can all be observed using DSC.

DSC has been used for quantifying an amorphous or crystalline phase in nanosolids from glass transition endotherm, crystallization exotherm, and fusion endotherm parameters.

Glass transition endotherm is a result of the presence of the amorphous phase, and the crystallization exotherm results from recrystallization of the amorphous content [113]. It is also used to determine the interactions between polymer and metal oxide NMs. The melting temperature of NPs is lower than that of the bulk material owing to a higher value of surface-by-volume ratio. For example, the pure polyvinyl alcohol (PVA) fibers shown in Figure 10 investigated that the endothermic peak below 100 °C represents the loss of absorbed water. The two exothermic peaks at about 305 °C and 500 °C, respectively, are associated with the degradation of the side chain and the scission of C-O and of the main chain and the scission of C-C. For the hybrid fibers, the exothermic peak below 450 °C is not observed, while the peak around 500 °C is sharp and strong, suggesting that the degradation of PVA is faster. From these DSC results, the existence of some interactions between a PVA molecule and ZnO *via* forming an H bond and an O-Zn-O bond have been studied [115].

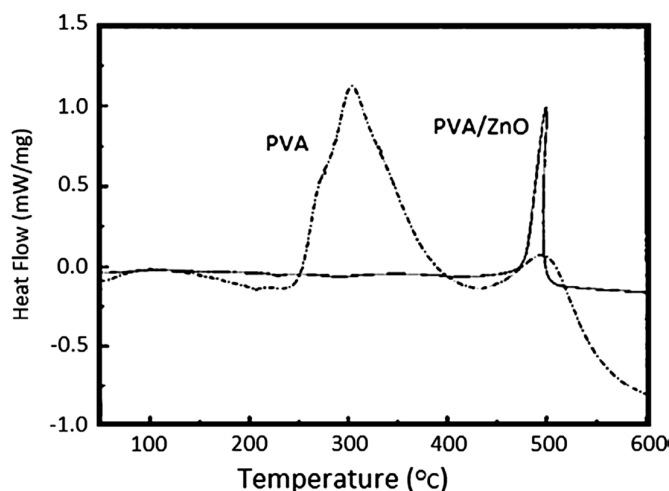


Figure 10. DSC curves of polyvinyl alcohol (PVA) and PVA/ZnO fibers, showing that PVA was stabilized by ZnO until heating to 450 °C [115].

2.5.9. Thermogravimetric analysis (TGA)

TGA describes the process in which the sample mass of a material is monitored with heating. Results the mass of sample will either increases or decreases as a result of changes in the material. The major instrument outputs are thermograms, either the % weight loss with time or the % weight loss with temperature to monitor changes in a material. Some of the most commonly observed transitions include loss of water (*i.e.*, desorption or vaporization) or oxidation (*i.e.*, uptake of oxygen from a reactive environment). Decomposition of the material, whether through combustion (an exothermic event in a reactive environment) or

pyrolysis (an endothermic event in an inert environment), is one of the most common chemical phenomena studied in TGA and express with the residual mass of the sample at a given temperature [116]:

$$M_{res} (\%) = \frac{M_{r,T}}{M_s} \times 100\% \quad (12)$$

where $M_{res} (\%)$ is the residual mass expressed as a percentage, $M_{r,T}$ is the mass remaining at as specific temperature (T) in grams, and M_s is the initial mass of the sample in grams.

2.5.10. Differential thermal analysis (DTA)

A similar technique to DSC is DTA which measures the difference in temperature between the sample and the reference, not the heat flow. The material being studied in DTA undergoes various thermal cycles (heating and cooling cycles), using an inert reference material. The record DTA, curve; the temperature difference (ΔT) should be plotted on the y-axis with endothermic reactions downward and temperature or time on x-axis increasing from left to right and its schematic representation is illustrated in Figure 11 [117]. In DTA, the temperature difference is measured in terms of microvolt which is equivalent to ΔT .

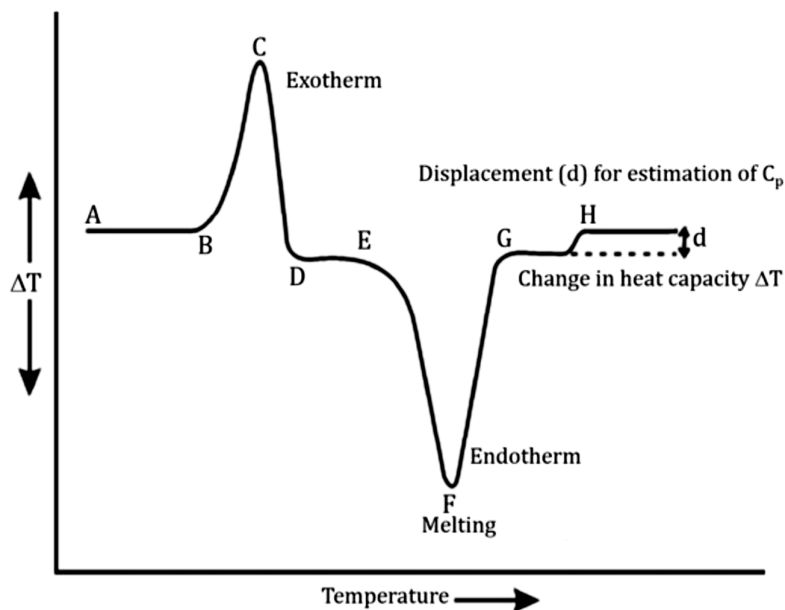


Figure 11. Schematic representation of components of DTA [117].

2.5.11. Cyclic voltammetry (CV)

CV is perhaps the most versatile electroanalytical technique in electrochemistry and used to study the chemical reactions of an electroactive species [118]. Its basic principle and routine

voltammetric experiments are performed using three-electrode configuration system with conducting supporting electrolyte. After scan it is represented by cyclic voltammograms shown in Figure 12: (a) the International Union of Pure and Applied Chemistry (IUPAC) convention that displays the negative (reducing) potentials to the left of 0.00 and positive (oxidizing) potentials to the right, where the cathodic (reduction) currents are displayed as negative (down) and anodic (oxidation) currents are displayed as positive (up); (b) the classical voltammogram (US convention), in which more negative potentials are located to the right and more positive potentials are on the left of zero value, where the cathodic and anodic currents are displayed as positive (up) and negative (down) values, respectively [118].

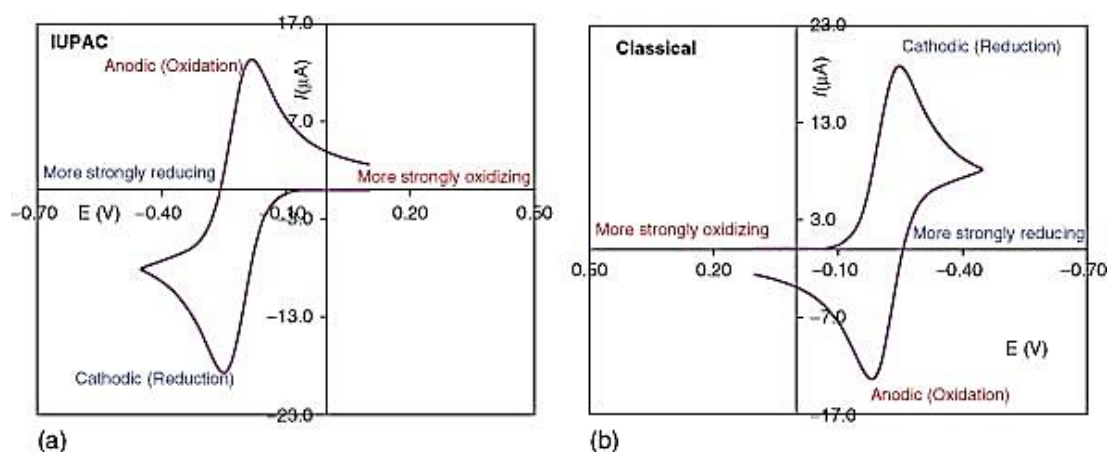


Figure 12. The IUPAC (a) and classical (b) presentations of cyclic voltammograms [118].

Electrode reactions: Thus a typical electrode reaction involves the transfer of charge between an electrode and a species in solution. The electrode reaction usually referred to as electrolysis, typically involves a series of steps [119]:

- Reactant (Ox) moves to the interface: this is termed mass transport
- Electron transfer can then occur between the electrode and reactant close to the electrode (typical distances are less than 2 nm).
- The product (Red) moves away from the electrode to allow fresh reactant to the surface.

2.5.12. Electrical impedance spectroscopy (EIS)

EIS is one of the main techniques that studies about electrodes kinetics, mechanisms of interfacial processes, and evaluation of parameters like double layer capacitance. In contrast

to other electrochemical techniques, it has an advantage to study the bulk as well as interfacial processes (electrolyte/electrode interfaces) connected with time constants ranging from minutes down to microseconds. Impedance extends the concept of resistance to alternating current (AC) circuits, and possesses both magnitude and phase, unlike resistance, which has only magnitude. The impedance is measured as a function of the frequency of the AC source in Hertz (cycles/s). Impedance: $Z = E/I$, Where E = Frequency-dependent potential and I = Frequency-dependent current. Consider, a purely sinusoidal voltage [120]:

$$E = E_0 \sin \omega t \quad (13)$$

where ω is the angular frequency ($\omega = 2\pi f$) and f is modulation frequency. The relationship between current (I) and voltage (E) represented sinusoidal signals with a separate phasor at the same frequency. As shown in Figure 13, their phasors are separated by a phase angle (ϕ). E is taken as a reference signal and ϕ is measured with respect to it and expressed as

$$I = I_0 \sin(\omega t + \phi) \quad (14)$$

where ϕ is a signed quantity, which is negative in this case.

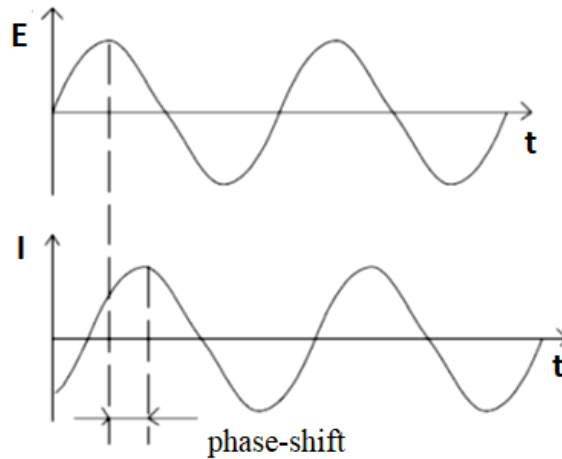


Figure 13. Sinusoidal current response in a linear system [120].

In general the angular frequency dependent impedance can be represented as

$$Z(\omega) = Z_{re} - j Z_{im} \quad (15)$$

where Z_{re} and Z_{im} are the real and imaginary parts of the impedance. For the example here, $Z_{re} = R$ and $Z_{im} = X_c = 1/\omega C$. The magnitude of Z , written $|Z|$ or Z , is given by

$$|Z|^2 = R^2 + X_c^2 = (Z_{re})^2 + (Z_{im})^2 \quad (16)$$

The phase angle, ϕ , is given by

$$\tan \phi = \frac{Z_{im}}{Z_{re}} = \frac{X_c}{R} = \frac{1}{\omega RC} \quad (17)$$

Therefore, in magnitude the impedance is a kind of generalized resistance. The presence of phase angle in impedance expresses the balance between capacitive and resistive components in the series circuit. For a pure resistance, $\phi = 0$; for a pure capacitance, $\phi = \pi/2$; and for mixtures, intermediate phase angles are observed.

Impedance: An expression according to Ohm's Law gave the impedance of the system can also be expressed as follows: So, impedance is expressed in terms of a magnitude, Z_o , and a phase shift, Φ .

$$Z = \frac{E_t}{I_t} = \frac{E_o \sin(\omega t)}{I_o \sin(\omega t + \phi)} = Z_o \frac{\sin(\omega t)}{\sin(\omega t + \phi)} \quad (18)$$

The impedance is represented as a complex number and can be written as follows:

$$Z(\omega) = \frac{E}{I} = Z_o \exp(j\Phi) = Z_o(\cos\phi + j\sin\phi) \quad (19)$$

From this expression EIS data may be displayed as either a vector or a complex quantity and $Z(\omega)$ is composed of a real and an imaginary part. The complex quantities are finally expressed in terms of Bode Plot or a Complex Plane (Nyquist) Plot. Nyquist Plot is when the real part is plotted on X-axis and imaginary part is plotted on Y-axis of a chart.

Admittance: Sometimes it is easy to analyze ac circuit in terms of admittance, represented as Y which is the inverse impedance, $1/Z$, and therefore represents a kind of conductance. A complex quantity usually symbolized by $Y = Y' + iY''$. It is the inverse of impedance and is sometimes called complex conductance [120]. The generalized form of Ohm's law can then be rewritten as

$$I = EY \quad (20)$$

These concepts are especially useful in the analysis of parallel circuits, because the overall admittance of parallel elements is simply the sum of the individual admittances. Vector relationship between Z and Y can be given as follows: If Z is written in its polar form, $Z = Z \exp(j\phi)$, then the admittance is

$$Y = \frac{1}{Z \exp(-j\phi)} \quad (21)$$

From this Y is a vector with magnitude of $1/Z$ and a phase angle equal to that of Z , but opposite in sign.

2.6. Electroactive microorganisms

In electrochemical reactions, special attentions have been given to bacteria due to its capability of interacting and transferring electron to solid conducting surface, as well as forming a slimy and slippery layer known as biofilm. They referred to as exoelectrogens while others referred to them as electrochemically active bacteria (EAB) and anode respiring bacteria. EAB in electronic application is an emergent field for reliable energy conversion device, biosensor, and bioremediation technology [121]. Since biofilms are complex microbial communities protected by an extracellular matrix composed of polysaccharides, proteins, and nucleic acids. This matrix improves biofilm cohesion and its adhesion to conducting electrode surfaces. The presence of organic compounds are used as a carbon source to survive bacteria life cycle [122].

EAB like *Escherichia coli*, *Shewanella putrefaciens*, *Geobacter sulfurreducens*, etc. can transfer electrons from inside the cell to extracellular acceptors via c-type cytochromes, biofilms, and conductive pili (nanowires) due to having coulombic efficiency and form a biofilms on the electrode surface that act as an electron acceptors and transfer directly to the anode for more energy production [18]. The bacterial transfer of electrons from the substrates (fed-source) to electrodes have two ways as shown in Figure 14 [18]. These are direct transfer (mediatorless) and indirect electron transfer (mediated). In mediated type dyes such as methyl red, thionine, methylene blue, and iron chelates are commonly added redox mediators to the reactor. To be effective, mediator should be able to penetrate cell membranes easily, able to grab the electrons from the electron carriers of the electron transport chains, should increase electron transfer from the metabolite, stable during long periods of redox cycling, and non-toxic to microbes.

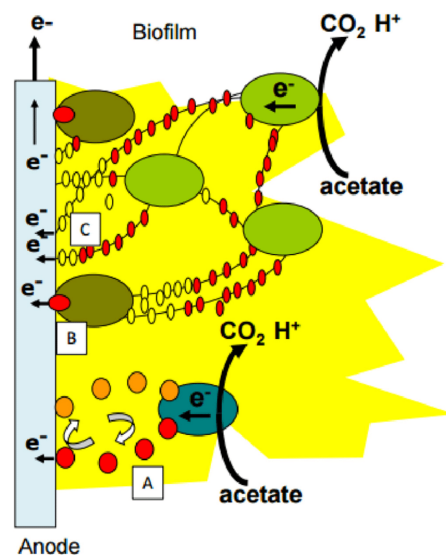


Figure 14. Electron transfer mechanisms: (A) Indirect transfer *via* mediators or fermentation products; (B) direct transfer *via* cytochrome proteins; (C) direct transfer *via* conductive pili [18].

Potentially, EAB can increase the open-circuit potential of anode immersed in a given liquid waste media of several hundred millivolts [123]. They harvest energy by reducing insoluble terminal electron acceptors including electrodes *via* extracellular electron transfer [124]. Bacterial strains from pure strains (*e.g.*, *E. coli*) and mixed cultures (*e.g.*, anaerobic sludge) were chosen for catalyst screening experiments. These criteria's are ability to anaerobically metabolize, non-stringent anaerobe, and non-pathogenic properties. *Escherichia coli* (*E. coli*) is gram-negative and rod-shaped bacterium that is facultatively anaerobic. Although some strains of *E. coli* are pathogenic, there are two commonly used laboratory strains, B and K12, which do not produce the toxic O antigen that confers pathogenicity to the other strains. Both of these strains are commonly used in industrial microbiology since they are well studied and easy to both culture and manipulate. Since, *E. coli* (*E. coli* K12 and *E. coli* B) is used in fermentation and other industrial processes since it is mostly non-pathogenic [125]. However, under special conditions, *E. coli* can be used to produce current. It has been reported to produce power densities as high as $3,800 \text{ mW m}^{-2}$, which is comparable to power production by *Geobacter* or *Shewanella* strains [126].

The pathogenicity of bacterial strains is important to consider especially when there is a possibility of future scale up. Pathogenic bacteria associated with fecal wastewaters and hospital wastes cause waterborne-disease outbreaks (typhoid and paratyphoid fever, salmonellosis, cholera, dysentery and other infections). It is therefore important to examine

the fate of pathogenic organisms in waste streams during the MFC-led power generation process as well as post-MFC treatment, to evaluate the risk of infections. Previous study have indicated that with $\text{pH} > 9.5$, oxidation-reduction potential, and the electron flow achieved in the anodic chamber of closed circuit MFCs could suppress the growth and deactivate for any pathogenic bacteria [127]. Finally, terminating the metabolic activity of anaerobic electro-active respiring microorganisms followed by stopping fuel substrate could be the best solution to remove the microorganism from its pathogenicity nature [128].

2.7. Role of anodes for MFCs

In MFC, anode plays a vital role for bacterial growth, removal rate, electron generation, and electron transformations to the cathode. Thus the fabrication of anode is a crucial step for providing a high surface area to the bacteria. Materials from graphene and its derivatives could provide a better outcome for anode in MFCs. However, the high cost of graphene sponge ($2000 \text{ \$/m}^3$) than graphite is economically unfeasible [129]. In addition, unmodified graphene material is not preferable due to its toxicity effect on bacteria through various mechanisms such as oxidative stress, membrane stress, and electron transfer. It can also physically damage the bacterial membranes by direct contact of its sharp edge [130]. On the other hand unmodified metal derivatives also suffer from corrosion in MFCs. Similarly anodes from metals and conducting polymers exhibit good performance in the form of composites. So, to reduce all limitations, anodes modified by composite materials are used effectively to enhance the working efficiency of MFCs. After modification, the large surface area and excellent conductivity of composite materials offers a favorable environment for stable relationship between bacteria and the anode materials [129]. Thus, in this dissertation, anode rather than cathode modification is considered more to achieve better outcomes in MFCs operations.

2.7.1. Metal oxide based anode

Metals and their oxide based materials are more selective than conventional carbon based anode materials due to the better conductive and electron movement facilitation properties. Metals such as gold, silver, titanium, and platinum are more conductive and corrosion resistance but they are very expensive to use. The other limitations of metal based anode (*e.g.*, stainless steel (non-corrosive)) are, they don't facilitate bacterial adhesion due to the smooth surface nature. A stainless steel grid used as an anode generated a higher current density than a plain graphite electrode. Noble metal-based anode electrodes help to decrease

the internal resistance in MFCs, but their higher cost and poor bacterial adhesion prevent their extensive integration during MFC operation. In addition, it is not easy to commercialize pure metal-based anodes in MFCs at a large scale due to their high cost and limitations [131].

The catalytic activity of metal oxide NPs (*e.g.*, TiO₂, ZnO, etc) and non-noble metal (*e.g.*, Zn, Cu, Al, Ni, etc) is equivalent to that of valuable metals, which can significantly decrease resistance and improve the attachment of bacteria on anode surface [129,132]. In general, anode materials must have a few basic properties to meet high-performance requirements, such as good biocompatibility, high conductivity, high chemical stability, good thermal and mechanical stability, and a large surface area [133].

2.7.2. Conjugated polymer and its composite based anode

Concepts from the polymer history evidenced that many polymers were well known in their non-conducting forms before their electrical properties were discovered. Later these concepts were completely changed to conducting polymers due their electrical conduction characteristics. As a result they are known as intrinsically conducting polymers and commonly called “organic semiconductors” or “synthetic metals”. However, in the history of conducting polymers, four major classes of semiconducting polymers have been developed, these include conjugated conducting polymers, charge transfer polymers, and ionically conducting polymers [134].

Conducting polymer from PANI, PPy, polythiophene, and so forth can be effective anode materials due to their high electronic conductivity characteristics. They are an excellent conventional carbon modifier [135]. These polymers play important role in increasing the surface area for microbes attachment to anode and a large number of active sites for nucleation [131]. From many literature reviews, PANI and PPy can penetrate into the cell membranes of bacteria and carry the electron through the metabolic pathway [25]. They are generally used as a substitute for high cost metal based materials to modify the anodic performances due to their comparative conductivity and lower cost. In addition, conducting polymer like PANI carry positive charges in the neutral experimental conditions which attract negatively charged bacteria for increasing in adhesion. Thus, compositing conductive polymer with carbon, metals/metal oxides, and their derivatives, could greatly enhance the working efficiency of anode electrodes [136].

For example, the brief electron transfer mechanism between PPy based composite materials act as promising anode electrodes due to the less charge transfer resistance and highly kinetic activity role as shown in Figure 15 [137]. The electron transport through reduction of metal oxides/PPy depicts great capacitance but a metal oxide alone has poor conduction. The possible mechanism of electron conduction through PPy generated from reduced metal oxides increases in conductivity. This was enhanced by introducing PPy conductive polymer. Since PPy exhibits a high degree of π - π^* conjugation, this favors the electron delocalization. The structure of PPy contains a quinoid ring in which nitrogen atom doped with polarons and bipolarons, which act as a charge carrier and responsible for electrical conductivity of PPy in the system. In the case of an oxidative state of PPy, the removals of electrons further lower the ionization energy. The lower energy leads to the formation of polarons and bipolarons, which fairly contribute to enhancing the conductivity, by being charge carriers with the same mobility. So, the incorporation of PPy with metal oxides is also very much important for conductivity and propagation of electrons. From the mechanism it demonstrates that the layer of PPy with metal oxides represented by M^{o+} where M is an arbitrary metal (Mn and Fe in this case) and (o+) defines the oxidation state of the metal moieties [137].

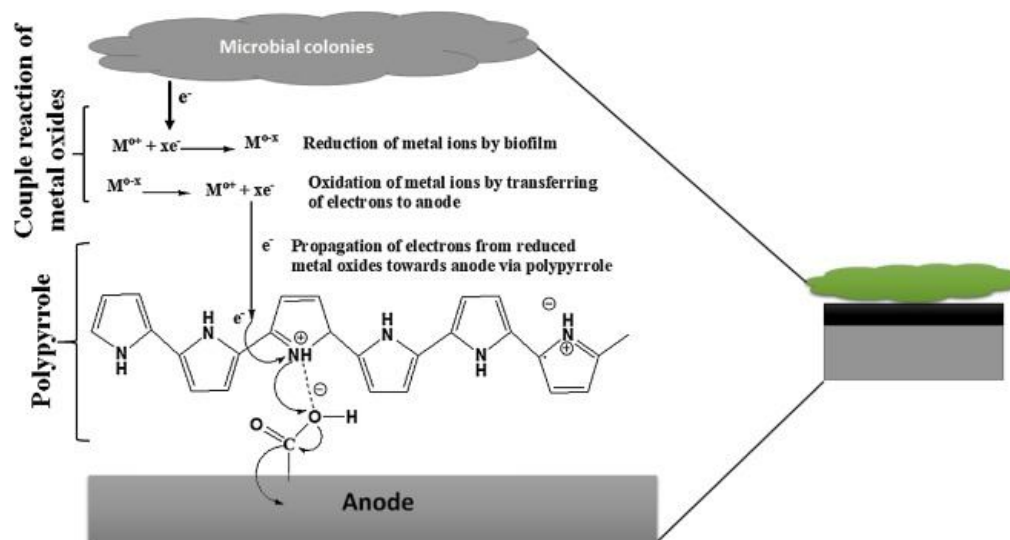


Figure 15. Electron propagation mechanism in PPy generated by reduction of applied metal oxides [137].

From the mechanism, the reduction of M^{o+} by x electrons is supported by bacterial colonies is a simultaneous process with oxidation of organics presented in the bioreactor. The reduced metal oxides in the form of (M^{o-x}) further transfer the electrons to PPy and regain

their previous oxidation states (*i.e.*, M^{0+}). The propagation of electrons from reduced metal oxides follows the path formed by polarons and bipolarons which make the transfer of electrons more efficiently to the anodes [137].

2.7.3. Anode performance for wastewater treatment and MFCs

The anode has a significant role for toxic pollutant removal and bioelectricity production in the presence of EAM as a biocatalyst. Bacteria are associated with the anode surface to generate electrons and protons during their respiration. The electrode provides a great enough surface to allow bacteria to grow and oxidize, as briefly described in Figure 16 [133]. The anode's performance provides MFCs with great electric output and the bioremediation of wastewater. The bioremediation of wastewater is considered to be a very efficient potential application of MFCs. Many conventional methods have been reported for wastewater treatment, but they are all associated with some drawbacks, such as high costs, a risk of self-toxicity, being difficult to operate, and remaining unstable for environmental safety.

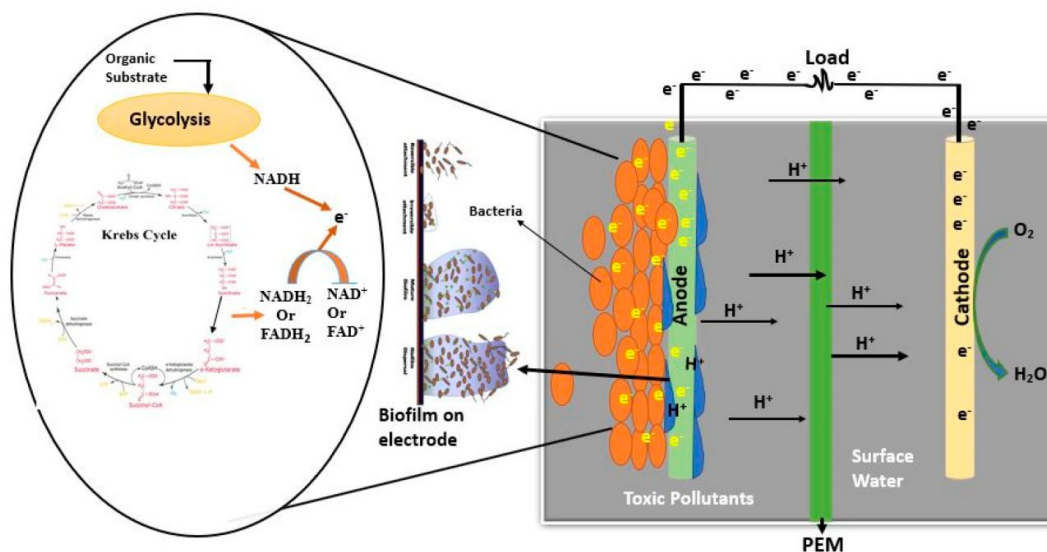


Figure 16. Working efficiency of anode electrode in MFCs [133].

The anode electrode in MFC system has a potential technique for the successful bioremediation of several types of wastewater, such as petrochemical industrial wastewater, swine wastewater, seafood processing wastewater, livestock waste, vegetable and food-processing waste, dairy wastewater, and surgical cotton industry waste [133]. In the case of organic pollutants, the oxidations of organic substances produces protons and electrons in the anode chamber with the help of exoelectrogens and degrade the toxic organic pollutants

in water. These protons are transferred to the cathode directly or through a membrane sources and the electrons moved through the outer circuit. This process depends upon the working efficiency of the electrodes. The electrodes provide a surface area to bacteria for their growth and respiration processes, which facilitate the transfer of electrons and protons.

Generally, the high-quality material could address many challenges that create disturbance during operation, such as long-term stability due to metal corrosion and solved by modifying anode with hybrid composite materials from conducting polymers and metal oxides. Therefore, in terms of wastewater treatment and energy production, the anode must be efficient and unique to achieve better results [133].

2.8. Applications of MFCs

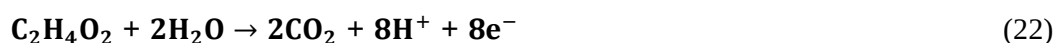
2.8.1. Bioelectricity production

The first concept of producing bioelectricity in the presence EAM was observed by Michael Cresse Potter in 1911 [138]. He discovered electrical energy from cell cultures of *Saccharomyces* and *E. coli* using platinum electrodes and seen in detail on MFC evolution [139]. Furthermore, Table 2 shows the detailed timeline of MFC demonstration [139].

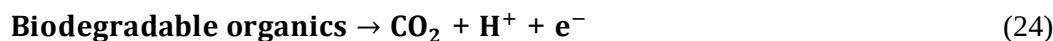
Table 2. History of discovery of bioelectrochemical systems [139].

Year	Scientists	Discovered issue
1791	Luigi Galvani (Physician and physis, Italy)	Bioelectric phenomenon.
1910	Potter, M. C. (Professor of botany at the university of Durham, U.K.)	Voltage generation and current delivery by microorganisms.
1911	Potter, M. C. (Professor of botany at the university of Durham, U.K.)	Discovered electrical energy from cell cultures of <i>Saccharomyces</i> and <i>E. coli</i> using platinum electrodes.
1931	Cohen, B. (Cambridge, U.K.)	Voltage generation and current delivery by microorganisms.
1962	Rohrback, G. H., Scott, W. R. and Canfield, J. H.	Designed the first biological fuel cell in which <i>Clostridium butyricum</i> was used as biomaterial to generate hydrogen by glucose fermentation.
1966	Williams, K. R.	Showed the rice husk is a potential source of lignocellulose as it can produce upon fermentation many useful enzymes and biofuels resulting in 40 mA at 6 V using biological fuel cells.
1993	Allen, R. M. and Bennetto, H. P. (Kings college in London, U.K.)	Demonstrated improved biological fuel cells using various microbes to increase both the reaction rate and the efficiency of electron transfer using mediator systems.
1999	Kim, H. J., Hyun, M. S., Chang, I. S., Kim and B. H. (Korean institute of Science and Technology, South Korea)	Discovered firstly that certain electrochemically active bacterial species use no mediator molecules.

The production of bioelectricity in an anaerobic bioreactor compartment found in the MFC device is the current strategy to facilitate renewable energy sustainability. The device uses EAM as a biocatalyst. The active biocatalyst found in the anode compartment oxidizes the carbon sources or substrates, and generates electrons and protons. As a further illuminating applications, taking anodic reaction of acetic acid and its decomposition chemical reaction as a model carbon source is presented in Equation 22. Oxygen in the anode chamber will inhibit the production of electricity; thus, a pragmatic system must be designed to keep the bacteria separated from oxygen (anaerobic chamber for anodic reaction). In the air chambered MFC, the anode chamber where the bacteria grow by oxidizing fuels like acetate while the cathode chamber where the electrons reacts with oxygen given in Equation 23.



In this regard, microorganisms play an important role in anode chamber and generated electrons. These generated electrons are utilized to reduce electron acceptors in cathode once they passed through external circuit. So, anaerobic anode compartment is responsible to degrade the biomass. This compartment is filled with substrate, mediator (it is optional), microorganism and the anode electrode as electron acceptor as it described from the general chemical reactions in the anaerobic environment (Equation 24).



It is necessary to point out that activation energy required for anodic reactions must be lowered by means of appropriate catalysts. It affects microbial electron transfer capacity in MFC. As a result it ensures it's theoretical (ideal electrode properties) futurity, such as good in electrical conductivity and low resistance, strong in biocompatibility, chemical stability and anti-corrosion, large surface area, and appropriate mechanical strength and toughness [140]. So, modification of anode electrode could be useful in promoting the performance of MFCs by governing the criteria. Thus MFC is fantastic technology in small electrical devices like LED lamps and digital clock [140].

2.8.2. Bioremediation

The demand for new technologies to accelerate the decontamination of contaminated sites and reduce the costs of these technologies is increasingly growing. Techniques used in the remediation of wastes fall under physical, biological, photolytic, chemical, and bioelectrochemical major categories. Of these, bioelectrochemical systems (BES) are

regarded as a promising alternative for common biological wastewater treatment technology. MFC is a typical BES which can remove organic pollutants through oxidation at anodes system [141].

The poor kinetics of reduction reaction at cathode chamber at neutral pH and low temperatures has hindered bioremediation improvement in MFC performances. Platinum is known to be the best catalyst for the reduction reaction in acidic and alkaline media, and is the most commonly used catalyst in the MFC [142]. However, platinum cost is too high in MFCs and finding platinum-free electrocatalysts represent a necessary alternative. Roche et al. [143] have thus investigated the performance of operating MFC using MnO₂ catalysts formed chemically on an activated carbon as cathode materials. The test was performed at neutral and alkaline pH [143]. Later, the electrocatalytic activity of MnOx modified with Cr³⁺, Fe²⁺, Co²⁺ [144], Zr⁴⁺ [145], and metal (*e.g.*, Ni, Mg) ion [143] have been performed for better bioremediation efficiency through doping. In such a way all these catalysts improves the catalytic activity towards the reduction reaction. Beyond hybridization with metals, electrodeposition of MnO₂ on polymers like PPy-coated stainless steel greatly improved MFC efficiency towards remediation with almost 100% COD removal followed by maximizing power density of 440 mW m⁻².

MFCs is also regarded as a form of a biosensor, where microorganisms in the anode chamber function as a biological recognition element, whereas proton exchange membranes and electrodes act as a transducer. The bacteria can sense the sudden present or a change in the level of target analytes and then give the response on its output electric current. The current generation is dependent on microbial respiration and proportional to the fuel concentration [146].

The main objective of MFCs-based biosensor is monitoring water quality in real time by optimization of the operating conditions, such as pH, organic loading rate, temperature, etc. So, its quantitative justification focus on the correlation between the signal output (*e.g.*, voltage, current) and changes in the level of target analytes (*e.g.*, BOD, COD, toxicity). The high sensitivity detection of the desired compounds is therefore one of the important targets of the MFC-based biosensor. It is generally influenced by the surface area of the anode as given in the Equation 25 [146]:

$$\text{Sensitivity} = \frac{\Delta I}{\Delta C \times A} \quad (25)$$

Where ΔI is change of the current output in μA unit, ΔC is change of the analyte concentration in mM unit, and A is surface area of the anode material (cm^2).

Thus, MFCs have been utilized as biosensor to measure the parameters like COD and other physicochemical parameters. The unique advantage of MFC based biosensor is that it can be worked without additional signal transducer or external power source. The basic principle and basic components are MFC based biosensors are shown in Figure 17 [146].

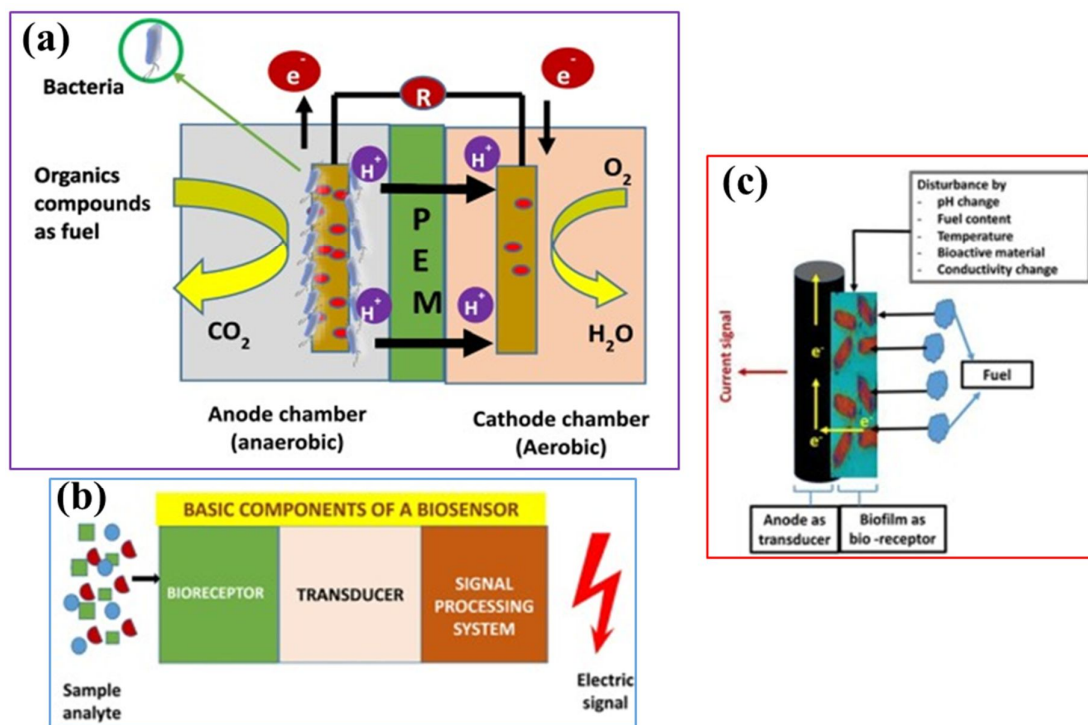


Figure 17. (a) Basic mechanism in MFC system, (b) components of a biosensor, and (c) basic principles of MFC as a biosensor [146].

3. MATERIALS AND METHODS

3.1. Materials and Instruments

3.1.1. Chemicals

KMnO₄ (99%, Alpha Chemika, India), nickel (II) chloride hexahydrate (NiCl₂.6H₂O ≥ 99.99%, Merck, Germany), aniline (99.5%, Merck, Germany), ammonium per sulfate (APS; (NH₄)₂S₂O₈) 98%, Fisher Scientific, India), ethanol (AR, Shantou, Guangdong, China), Methyl red (99.8%, Alpha Chemika, India), and HCl (37%, Merck, Germany) were used as received. *V. amygdalina* leaf was obtained from Adama Science and Technology University, Adama, Ethiopia (latitude 8°33'43.56" North and longitude 39°17'23.28" East). Nutrients, substrates, and chemicals used in MFC operations were analytical reagents and used as received without further purification. Distilled water was used for all experimental run.

3.1.2. Instruments

A double beam UV-Vis spectroscopy (Azzota SM-1600 SPECTROPHOTOMETER, USA) was used to elucidate and measure the optical properties of synthesized materials in the range of 200-800 nm. Thermogravimetric-differential thermal analyses (TGA-DTA) were carried out on a DTG-60H detector (Shimadzu, Japan), with the sample flow rate at 50 mL min⁻¹ under nitrogen (N₂) gas. Differential scanning calorimeter (PerkinElmer, DSC 4000, USA) was carried out under N₂ at a flow and heating rates of 20 mL min⁻¹ and 10 °C min⁻¹, respectively. The crystalline structure of the synthesized α-MnO₂, PANI, α-MnO₂/NiO, α-MnO₂/PANI, α-MnO₂/NiO/PANI materials was confirmed using X-ray diffractometer (XRD-7000, Shimadzu, Tokyo, Japan) with a voltage of 40 kV and a current of 30 mA at (Cu Kα = 1.5406 Å) radiation as an X-ray source in the 2θ range from 10 to 80°. The crystallite size of the NMs was found using the Scherrer's formula. FTIR spectra of the prepared samples were recorded with Fourier Transform Infrared Spectroscopy (FTIR, Perkin Elmer 65, PerkinElmer, Inc., Waltham, USA) in the range 4000-400 cm⁻¹ with samples prepared using KBr pellets. SEM was performed using a JEOL Ltd. instrument with a model of JCM-6000Plus and SEM-EDX (EVO 18, ALTO 1000, Carl Zeiss, Germany) to observe the structural morphology of all synthesized materials. After importing the SEM image of prepared materials to the Gwyddion software [147,148], display of 2D and 3D image, the height distribution, surface roughness, and pore structure of the material surface was distinguished, calculated, and recorded.

3.2. Collection of plant leaf

A fresh leaf of *V. amygdalina* was collected from compound of Adama Science and Technology University, Adama, Ethiopia shown on Figure 18a. The collected plant leaf was washed three times with distilled water and air dried in the shade until the moisture content was completely removed. The dried leaf (Figure 18b) was grounded to reduce its size physically using mortar and pestle to tiny particles (Figure 18c) and stored in polythene bags until used.



Figure 18. (a) Fresh, (b) Dried, and (c) Powdered leaves of typical *V. amygdalina* plant.

3.3. Preparation of plant extract

Vernonia amygdalina plant collection and aqueous extraction was done by following the literature with little modification [149]. In brief, the fresh plant leaf was washed three times with distilled water, exposed to air and dried until the moisture content was removed. The dried leaf was grounded using mortar and pestle to tiny particles and stored in polythene bags until used. 10 g of dried powder of *V. amygdalina* was boiled in 200 mL of distilled water with stirring for 15 minutes. A dark brown color extract solution was obtained as shown in Figure 19a. The extract was allowed to settle for 30 minutes at room temperature. Then, the clear extract was collected by filtration using Whatman no. 1 filter paper (see Figure 19b), and centrifuged at 4000 rpm for 15 minute. Finally, the extract was collected in a colored bottle and stored at 4°C until further uses.

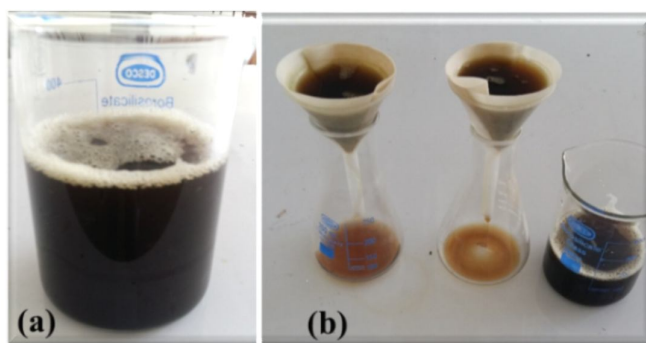


Figure 19. (a) Aqueous extracted and (b) filtration of *V. amygdalina*.

3.4. Synthesis of materials

3.4.1. Biosynthesis of MnO₂ NPs

Design of experiment for the biosynthesis of MnO₂ NPs was obtained by D-optimal design response surface approach by using Design-Expert 11 (version 11.0.5.0). The choices of an optimal design with these approach was chosen as the best recommended model due to its simplicity and building the precise fitting for long independent factors [150]. Biosynthesized MnO₂ NPs was prepared by following the reference with little modification [151]. In brief, 25 possible combination as shown in Table 3 were measured by dissolving different aqueous solutions of initial KMnO₄ concentration (0.5, 1, 1.5, and 2.5 mM) at different pH values (4, 5, 6, and 8). Then, different volume ratios (20, 30, 40, and 60%) of *V. amygdalina* crude extract were added to the solutions and stirred at room temperature for various length of time (45, 60, 75, and 105 minute). Each of the sample precipitates was obtained by centrifuging at 4000 rpm for 15 minutes. Finally, the obtained NPs were washed with distilled water and ethanol several times as shown in Figure 20, and the washed samples were suspended in 10 mL distilled water for UV-Vis spectrophotometric measurements for optimization. The design expert software was used to evaluate the overall variance analysis (probability < 0.5) at about 95% confidence interval to obtain the superior yield of MnO₂ from the measured absorbance. Such better and higher yields in terms of responses were stated using the fitted non-linear second order polynomial (Equation 26) [152].

$$Y = \beta_o + \sum_{i=1}^n \beta_i X_i + \sum_{i=1}^n \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^n \beta_{ij} X_i X_j \quad (26)$$

Where Y is the predicted response (dependent variable), X_i , X_i^2 , and X_j are experimental factors; β_o is the model constant; β_i is the linear effect; β_{ii} is the squared effect; β_{ij} is the variable interaction effect, and n is the number of levels and for these study four levels were chosen. For our work a total of four factors are mentioned to optimize them.

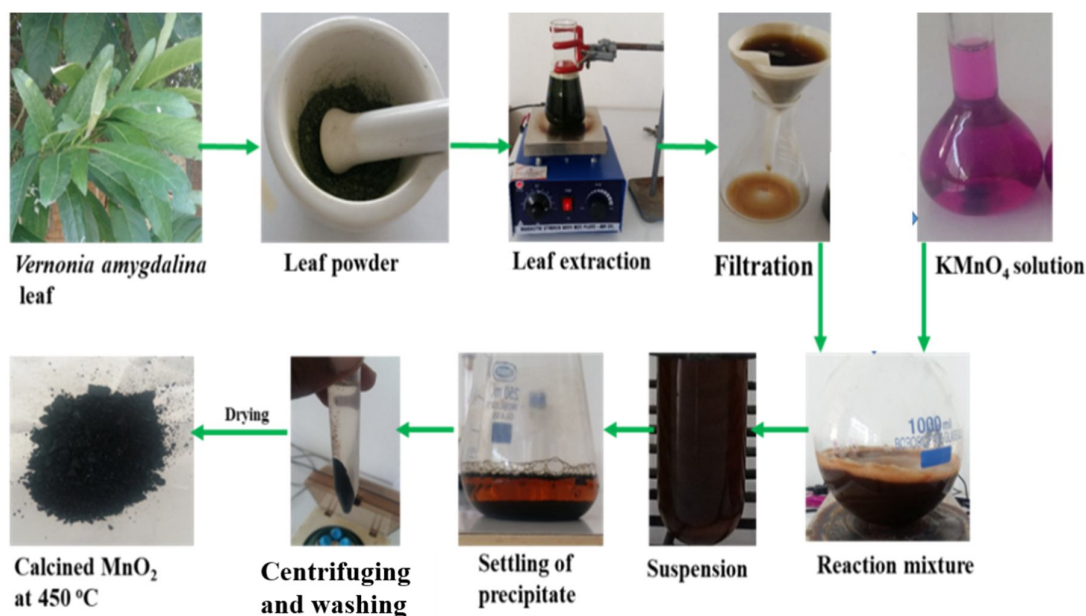


Figure 20. Schematic diagram for the green synthesis protocol of α - MnO_2 NPs.

An optimized biosynthesized α - MnO_2 NPs was prepared as follows. Briefly, ~ 56% aqueous solution of ~ 1.8 mM KMnO_4 and ~ 44% *V. amygdalina* leaf extract by volume were mixed and stirred at room temperature for ~ 104 minutes. The pH of the performed mixture was 6.02. The black brown suspension was formed and centrifuged at 4000 rpm for 15 minutes. The precipitate was collected and washed several times with distilled water and ethanol to remove impurities. The final precipitate was collected and dried overnight at 80 °C. Finally, the sample was calcined at 450 °C for 3 hours. Since ~450 °C was selective as a stabilized temperature from TGA curve as shown in Appendix, Figure A1a.

Table 3. Response surface optimal design model for MnO₂ NPs absorption results.

Run	Factor 1 A: Extraction (%)	Factor 2 B:Permanganate ion (mM)	Factor 3 C:pH -	Factor 4 D:Time (Minutes)	Absorbance (320 nm)		
					Actual (non-transform)	Actual (transform to square root)	Predicted (transform to square root)
1	60	2.5	8	45	0.967	0.9834	0.9343
2	60	2.5	8	105	0.985	0.9925	1.02
3	20	1	4	105	0.212	0.4604	0.4908
4	60	1	5	75	0.263	0.5128	0.6090
5	20	2.5	5	75	1.056	1.03	0.9609
6	40	1.5	6	75	0.724	0.8509	0.8667
7	40	1.5	8	75	0.738	0.8591	0.8570
8	20	1.5	8	105	0.823	0.9072	0.9293
9	60	0.5	8	75	0.011	0.1049	0.1230
10	30	2.5	4	45	1.046	1.02	1.06
11	30	2.5	8	45	1.051	1.03	1.12
12	30	0.5	6	45	0.022	0.1483	0.1720
13	30	0.5	6	75	0.029	0.1703	0.1505
14	40	1.5	6	75	0.832	0.9121	0.8667
15	60	1	6	45	0.314	0.5604	0.5640
16	20	1	4	45	0.387	0.6221	0.5155
17	40	2.5	4	105	1.054	1.03	1.03
18	60	0.5	4	45	0.034	0.1844	0.1723
19	60	2.5	4	45	1.156	1.08	1.07
20	40	1.5	8	75	0.862	0.9284	0.8570
21	20	2.5	8	45	1.371	1.17	1.15
22	20	1.5	8	105	0.886	0.9413	0.9293
23	20	0.5	8	45	0.043	0.2074	0.2070
24	20	1	4	60	0.132	0.3633	0.4638
25	60	0.5	4	105	0.178	0.4219	0.3658

It can be seen from UV-Vis absorption spectra of each run presented as shown in Figure 21 the absorption intensity was increased and depends on the concentration of the MnO₂ NPs. An increase in the absorption intensity indicated the better in solubility and dispersion of the synthesized MnO₂ NPs in solution [153].

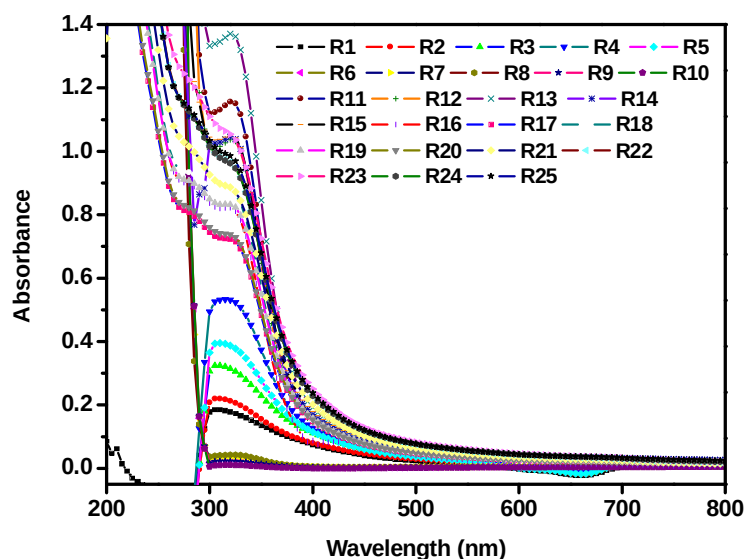


Figure 21. UV-Vis absorption spectrum of 25 experimental run for biosynthesized α -MnO₂ NPs (R stands for experimental run).

3.4.2. Biosynthesis of NiO NPs

20 mL of *V. amygdalina* plant leaf extract was mixed with 100 mL of 0.116 M of aqueous NiCl₂.6H₂O solution was added drop wise with constant stirring at 90 °C for 40 minutes [154], which leads to the volatilization of water and combustion of the reaction mixture. The separated black powder was dried at 100 °C in hot air oven for 1 hour and calcined in a muffle furnace at 450 °C for 2 hours [155].

3.4.3. Biosynthesis of MnO₂/NiO binary composites

In the typical biosynthesis, the procedure is adopted from the literature [88], with little modifications (designing, analysis, and observation). In brief, 5 mM and 10 mM aqueous solutions of NiCl₂.6H₂O to 5 mM and 10 mM of KMnO₄ with a concentration ratios of 33.34, 50, and 66.67%, respectively) were dissolved in double distilled water. The formed solution was continuously mixed under round bottom flask placed on a controlled hot plate stirring at a fixed temperature and pH of 80 °C and ~8, respectively (see Figure 22). After 15 minutes, pure leaf extract with different concentration ratios (40, 60, and 80% v/v) were

added drop wise in the solution. The reaction mixture was kept for 15, 45, and 75 minutes for stirring until thick dark brown formation with reduced stirring speed was formed. The obtained precipitate was centrifuged at 4000 rpm for 15 minutes. The precipitate was collected and washed several times using distilled water and absolute ethanol to remove organic contaminants. Portion of the washed precipitate was suspended in 5 mL distilled water for UV-Vis spectra analysis. The three factors with three levels of process variables and nine experimental runs were designed to assess extract ratio effect (v/v), nickel ion concentration ratio, and reaction time (Table 4), the formation of α -MnO₂/NiO nanocatalyst and measured optical band gap energy change was monitored using UV-Vis spectroscopy scan.

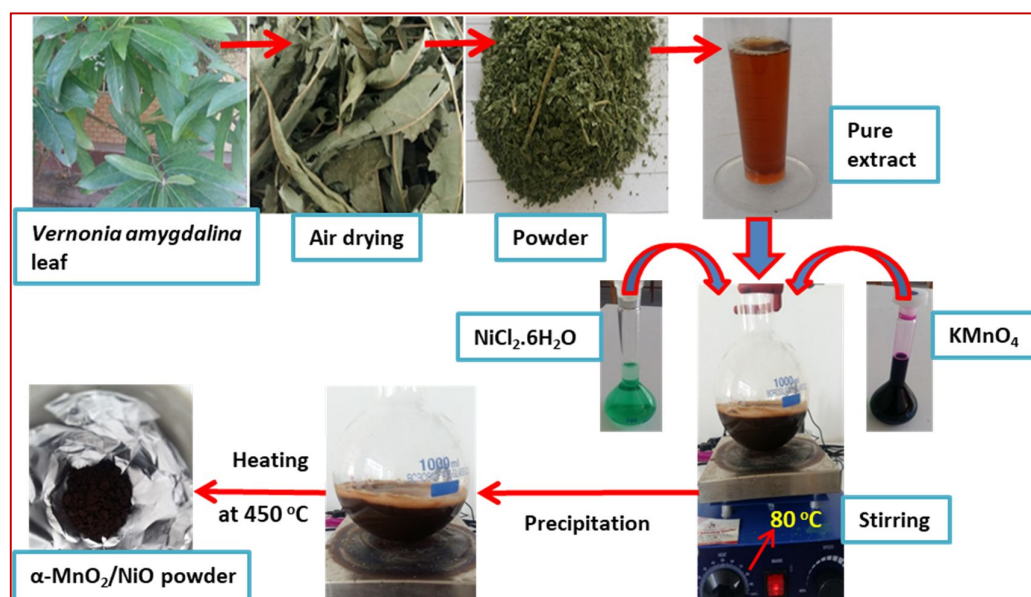


Figure 22. Schematic diagram of green synthesis protocol for α -MnO₂/NiO bimetallic oxide catalyst.

An optimized MnO₂/NiO binary composite was prepared as follows. Briefly, an aqueous nickel ion concentration of ~51% (v/v) to KMnO₄ was added in a round bottom flask which was placed on controlled hot plate at a fixed temperature of 80 °C and fixed pH = 8 with continuous stirring. After 15 minutes ~75% (v/v) of *V. amygdalina* leaf extract was added drop wise in the mixture. The reaction mixture was waited for ~ 25 minutes, stirring and then a thick dark brown precipitate was formed. The speed of the stirrer was reduced due to thick dark brown precipitate formation. The obtained material was centrifuged at 4000 rpm for 15 minutes. Recovered precipitates were washed several times using distilled water and absolute ethanol to remove organic contaminants. In the same manner, portion of the washed

precipitate was suspended in 5 mL distilled water for UV-Vis spectra analysis while the rest of the resultant precipitate was dried in hot air oven for 4 hours and calcined at 450 °C (this temperature was also selected as a stable temperature from TGA curves as shown in Appendix, Figure A1b for 2 hours to give α -MnO₂/NiO bimetallic oxides.

Table 4. Experimental planning of I-optimal coordinate exchange design and absorption edge (nm) response for MnO₂/NiO sample at 95% confidence interval.

Run	Factor 1	Factor 2	Factor 3	Response 1	
	A: Extract ratio (%)	B: Nickel ion concentration (v/v%)	C: Time (min)	Absorption edge (nm)	
				Actual Value	Predicted Value
1	40	33.34	15	369.70	371.36
2	40	50	45	372.90	372.56
3	40	66.67	75	366.00	367.66
4	60	33.34	45	348.50	346.52
5	60	50	15	396.70	394.72
6	60	66.67	75	389.00	387.02
7	80	33.34	75	386.60	387.26
8	80	50	45	380.00	381.64
9	80	66.67	15	381.50	382.17

3.4.4. Synthesis of PANI

The synthesis of PANI was based on mixing aqueous solution of aniline hydrochloride and Ammonium per sulfate (APS) at room temperature, followed by the separation of PANI hydrochloride precipitate by filtration and drying as described from the literature [156]. Briefly, 0.5 mL aniline monomer was dissolved in 1 M (100 mL) HCl aqueous solution in a round-bottom flask and stirred for 30 minutes. Next, 5.71 g of APS was dissolved in 50 mL of distilled water. Both solutions were kept separately for 1 hour at room temperature. Then, the two solutions were mixed in a beaker and stirred with a magnetic stirring until the color change from colorless to dark green, which indicates the formation of PANI, and kept for 4 hour to polymerize. The PANI precipitate was collected by filtration and washed with 0.2 M HCl solution until the filtrate become clear, then with ethanol several times to remove monomer, oligomer as well as with distilled water several times to remove the acid. The PANI powder was then dried in an oven at 60 °C for 24 hours.

3.4.5. Synthesis of α -MnO₂/PANI nanocomposite

A typical in-situ oxidative polymerization method was used to synthesis α -MnO₂ based PANI nanocomposites by following previously reported methods with little modification

[157]. Firstly (protonation step), 0.5 mL aniline (0.1 M) monomer was dissolved in 1 M HCl (100 mL) aqueous solution in a round-bottom flask and stirred for 30 minutes to form aniline hydrochloride. Secondly (homogenization of oxidant), different mass fraction of biosynthesized α -MnO₂ NPs was dispersed in 25 mL distilled water using 5 minutes ultrasonication time. Finally, a homogeneously suspended α -MnO₂ NPs was added drop wise to aniline hydrochloride solution with 2.86 g of 50 mL APS (oxidant and co-dopant) at room temperature, and the mixture was stirred continuously at room temperature for 4 hours to completely polymerize. The reaction mixture was turned into dark green precipitate, the obtained materials was then collected by filtration and washed with 0.2 M HCl solution until the filtrate become clear, then with ethanol several times to remove monomer, oligomer as well as with distilled water several times to remove the acid. Finally, the product was dried in oven at 60 °C for 24 hour to give α -MnO₂/PANI nanocomposite as shown in Figure 23. The composition of α -MnO₂ based composite is given in Table 5.

Table 5. Composition of α -MnO₂ composite.

Sample	α -MnO ₂ (wt%)	Aniline (v/v%)	Oxidant/Reductant
α -MnO ₂	100	0	<i>V. amygdalina</i> * (44% in volume)
PANI	0	100	APS ⁺ (5.71 g)
α -MnO ₂ /PANI	50	50	APS ⁺ (2.86 g)
α -MnO ₂ /PANI	67	33	APS ⁺ (2.86 g)
α -MnO ₂ /PANI	75	25	APS ⁺ (2.86 g)

*Reductant, ⁺ Oxidant

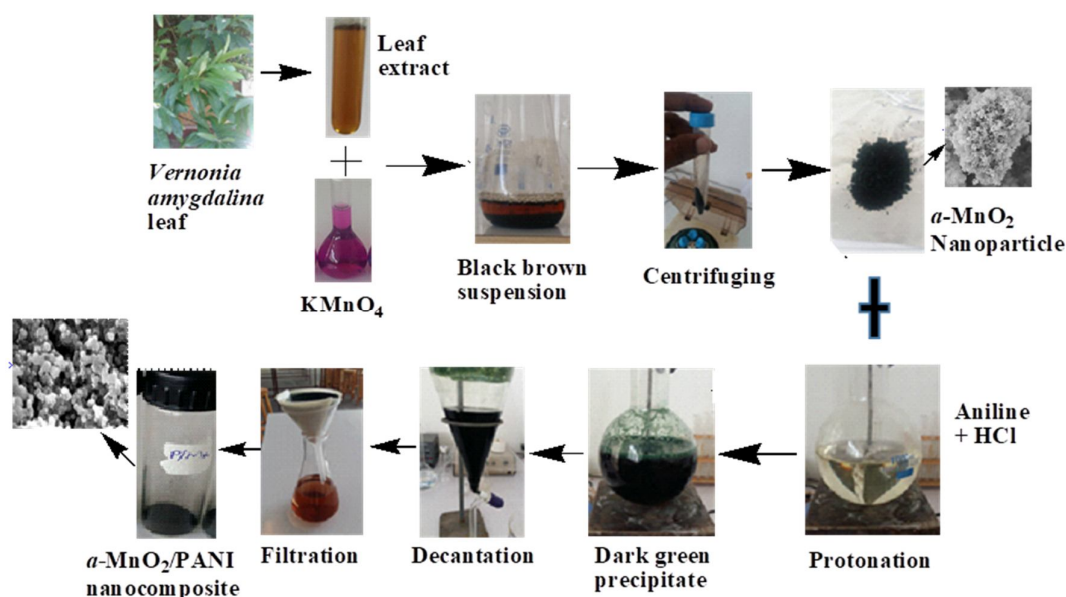


Figure 23. Synthesis protocol schematic diagram for α -MnO₂/PANI nanocomposite preparation.

3.4.6. Synthesis of α -MnO₂/NiO/PANI ternary composites

A typical in-situ oxidative polymerization method was used to synthesis α -MnO₂/NiO bimetallic oxide based PANI nanocomposites by following previously reported methods with little modification [157]. Firstly, a 0.5 mL of aniline (0.1 M) monomer was dissolved in 1 M HCl (100 mL) aqueous solution in a round-bottom flask and stirred for 30 minutes to form aniline hydrochloride. Secondly, 0.5 g of optimized biosynthesized α -MnO₂/NiO bimetallic oxide was dispersed in 25 mL distilled water using 5 minutes ultrasonication time. Finally, a homogeneously suspended α -MnO₂/NiO bimetallic oxide was added drop wise to aniline hydrochloride solution with 1.43 g of 50 mL APS (oxidant and co-dopant) at room temperature, and the mixture was stirred continuously at room temperature for 4 hours to completely polymerize. The reaction mixture was turned into dark green precipitate, the obtained materials was then collected by filtration and washed with 0.2 M HCl solution until the filtrate become clear, then with ethanol several times to remove monomer, oligomer as well as with distilled water several times to remove the acid. Finally, the product was dried in oven at 60 °C for 24 hour to give α -MnO₂/NiO/PANI composite.

3.5. Catalyst modified anode preparation

Before modification, pencil grade graphite electrodes (0.2 cm in diameter with 1 cm length, HB, Camlin, India) were soaked in distilled water for 24 hour and rinsed with 0.2 M PBS [158]. Afterwards, equal mass of synthesized α -MnO₂, NiO, PANI, α -MnO₂/NiO, α -MnO₂/PANI, and α -MnO₂/NiO/PANI samples with polyvinyl alcohol (PVA, M.W. = 44.05 g mol⁻¹) polymer binder solution were prepared by drop-cast coating method as follows. The purpose of the binder is to increase the bonding force between the prepared catalyst and bare PGE. Required amount of NPs were added in to the beaker; then it was kept in an ultrasonic water bath for 15 minutes under ambient condition to form a uniform solution since the binder step was adopted from reference [159]. The PVA was added in the ratio of 1:5 (10 mg, PVA to 50 mg, NPs) and dissolved in 10 mL deionized water, followed by mixing for 1 hour by magnetic stirring at 70 °C. Before coating on electrode surface, the mixture was kept for sonication to make uniform distribution of nanocatalyst throughout the solution. 100 μ L of uniformly distributed nanocatalyst based suspension was coated on PGE surface by drop-cast method with the help of microsyringe [160], and then it was dried for 12 hours at 60 °C using oven drying to improve its interface stability and bonding force of the modified layer.

3.6. Electrochemical characterizations

Electrochemical characterizations were performed by using bare/modified PGE, Ag/AgCl and Pt wire as a working, reference, and counter electrodes, respectively. CV measurements of bare and catalyst modified electrodes were recorded in potential window between -0.8 V and +0.8 V at scan rate of 50 mV s⁻¹. EIS measurements were recorded over the frequency range 200 kHz to 10 mHz using a sinusoidal excitation signal with amplitude of 10 mV superimposed on working potential ($E_{WE} = +0.252$ V versus Ag/AgCl electrode). In all electrochemical measurements, a 100 mM phosphate buffer solution (PBS, pH = 7.4) was used as a supporting electrolyte.

3.7. Microorganism inoculation

Effluents of wastewater sample were collected from Adama Science and Technology University wastewater treatment plant, Adama, Ethiopia, using glass bottle tied up with light weight steel round stick. The sample was immediately transferred to biology laboratory room and was kept overnight in a refrigerator at 4 °C. Areas which were used for culturing were cleaned with 95% ethanol during sample container opening. Before culturing, all the necessary facilities were sterilized in an autoclave at 120 °C for 30 minutes. After keeping at room temperature, 3 mL of settled waste sewage effluent sample was cultured in 400 mL autoclaved conical flask using Lactose broth as a culture media. After 15 minutes, 1 mL supernatant was taken from the conical flask with the help of micropipette and added the supernatant into another sterilized test tube in order to make dilution. The cultured sample was anaerobically incubated at 37 °C for 24 hours [161].

3.8. Isolation and characterization of *E. coli*

Isolation of coliforms were carried out with serial dilution technique and the plates were incubated at 37 °C for 24 hours [161]. The pure culture of the unknown bacteria isolated from effluents of wastewater sample was subjected to gram staining and morpho-biochemical tests/observations based on Bergey's Manual of Determinative Bacteriology. Careful and systematic techniques of strategy should be used to narrow down the types of bacteria that are present in an unknown wastewater sample [162]. So, Bergey's Manual of Determinative Bacteriology was used as a guide in the identification of the unknown bacteria. After 24 hours of incubation on nutrient agar at 37 °C the isolated coliform showed large, circular, convex, grayish white, moist, smooth, opaque and light pink colonies. The other characteristics of this coliform was also gram negative, rod shaped, mesophilic,

facultative anaerobic, lactose, and glucose fermenter with the production of acid and gas, indole positive, catalase positive, urease negative, and do not utilize citrate as carbon source. Finally, the identified and its morpho-biochemical characterized image was captured from Laboratory Biological Microscope (Trinocular with Total Magnification Upto 1500x With Optional Eyepiece WF15x) that showed in Figure 24.

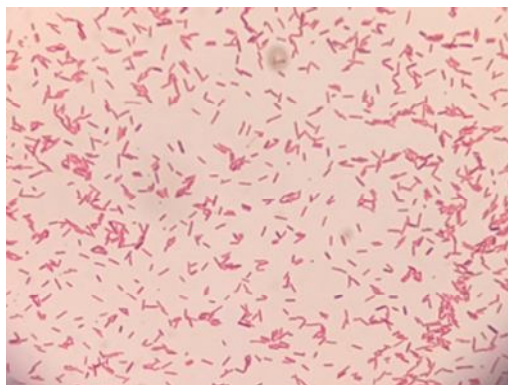


Figure 24. Gram-staining image of *E. coli*.

3.9. MFC setup and operation

A doubled chambered MFC setup was constructed from two equal glass Büchner flasks with volume size of 250 mL connected with a salt bridge with a Pyrex glass tube (9 cm length and 1.1 cm diameter) as shown Figure 25. The salt bridges were prepared by following the literature [163]. Briefly, 3 g of agar was added in 100 mL boiled water, and stirred for until dissolution. Then, 12 g of KCl was added over it. Since one end of the compression fitting was securely covered with aluminum foil. Finally, it allowed for cooling nearly 2 hours. The salt bridge was thus ready for use. Bare PGE was used as a standard working electrode and its modified with all nanocatalysts were used as anode while bare PGE was used as cathode. The anodic bioreactor was filled with artificial wastewater contained from 100, 160, and 166 mg L⁻¹ of glucose, 5840 mg L⁻¹ of NaCl, 100 mg L⁻¹ of KCl, 250 mg L⁻¹ of NH₄Cl, 12000 mg L⁻¹ of Na₂HPO₄·12H₂O, 2570 mg L⁻¹ of NaH₂PO₄·2H₂O, 840 mg L⁻¹ of MgCl₂·6H₂O, 500 mg L⁻¹ of FeCl₃, 2 mg L⁻¹ of MnSO₄·H₂O, 5 mg L⁻¹ of CaCl₂, and 340 mg L⁻¹ of yeast extract to fulfill the micronutrient deficiency [164]. The anode chamber was inoculated with *E. coli* and kept airtight by complete filling with glucose substrate as a carbon source with the inoculum added to achieve anaerobic conditions [165]. Before operations, pH and temperature parameters were optimized. The cathode chamber was filled with 50 mM KMnO₄ which was dissolved in a neutral medium (100 mM, pH 7.40) PBS.

Finally, the MFC operation was operated at room temperature (28 ± 2 °C). During the time of operation the anode chamber was containing with MR dye to visualize decolorization and the chamber was covered with aluminum foil to protect it from light illumination.

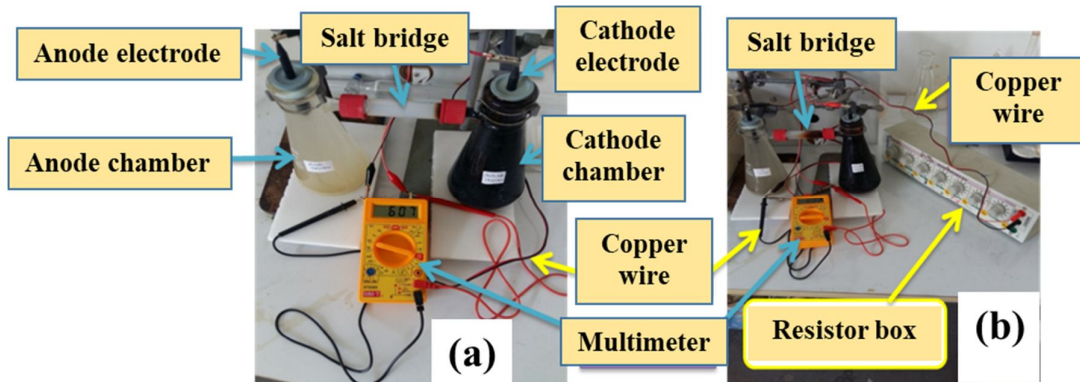


Figure 25. Photograph of Double chambered MFC experimental setup for (a) steady state condition and (b) non-steady state condition.

3.10. Measurements of electrochemical parameters

The circuit voltage measurement was recorded by a digital multimeter (DT830D, Haoyue, China) after 15 minutes stabilization for every 15 minutes continuous interval. Since the accuracy of the multimeter for voltage measurement is $\pm 1.2\%$ of indicated values ± 5 least-significant digits from its specification. Then by connecting it between an anode and a cathode the data for the polarization curves were measured from circuit voltage against external resistors ($100 \text{ k}\Omega$ - $100 \text{ }\Omega$) for 15 minute interval using Decade Resistance Box (ANSHUMAN, Model: DRB-7T). The current flow (I) across each external resistor was calculated *via* Ohm's law: $I = V/R$, V as a cell voltage and R as the external resistance (R_{ext}). Similarly, power P is derived as the product of voltage (V) and I. Current (J) and power densities (p) are calculated by the ratio of current and power per surface area of anode (area = $2\pi r \left(h + \frac{r}{2}\right)$ where h is height, r is radius, and π is 3.14 and for $\text{PGE}_{\text{area}} \approx 0.66 \text{ cm}^2$), respectively. Internal resistances (R_{int}) in each voltage drop during power density measurement was determined by fitting the slope of the polarization curve [166].

To explore the influence of different anodic pH on the operation MFC, the system was studied at five different pH of acidophilic (pH 4.45 and 5.52), near neutral (pH 6.47 and 7.42), and alkaline (pH 8.67) medium. The pH of the medium was adjusted to these pH values through addition of 0.1 M HCl and 0.1 M NaOH solutions. The effect of temperature on the doubled chamber bioreactor was studied with varying temperatures (25, 30, 35, and

40 °C) and produced current was monitored to adjust the optimum operation temperature from the results. All operations were monitored on bare PGE in every 15 minutes time interval for 3 hours at 500 Ω of load resistance with 100 mg L⁻¹ glucose.

SEM (measured using a JEOL Ltd. instrument with a model of JCM-6000Plus was used to observe the structural morphology of the formed biofilm. The characterization procedures are given as follows. First, anodic electrodes after a test including bare PGE were fixed in 2.5% glutaraldehyde for 2 hours. Then they rinsed three times using PBS (pH of 7.4, 100 mM) followed by dehydration at different alcoholic series (60%, 70%, 80%, 90%, 95% and 100%). Then all electrodes were air dried. Finally, anodes were subjected to SEM observations.

3.11. COD, CE, and DE measurements

COD measurements were conducted using acidic permanganate (COD_{Mn}) method under the protocol of standard methods [167]. Briefly, 10 mL aqueous sample solution from anodic bioreactor was transferred into a 250 mL conical flask; the sample was added with 1 mL of 9 M H₂SO₄ followed by 10 mL of 0.01 M KMnO₄ digestion solution. The sample was heated for 30 minutes in a water bath at ~96 °C. After digestion, the sample was cooled immediately in an icebox to quench the reaction. The remaining KMnO₄ in the sample solution was determined by spectrophotometer in the range of 200 nm-800 nm. A spectrophotometer is needed to make a standard calibration curves shown in Appendix, Figure A2a and b by measuring concentration of the remaining permanganate and their absorbance which was adopted from dichromate method [168]. If the sample actual COD was higher than the range, the sample was diluted but the real results were multiplied by dilution multiple [15]. The degradation efficiency was then evaluated by calculating the COD removal with the following formula:

$$\text{COD} = \left(\frac{\text{COD}_{\text{in}} - \text{COD}_{\text{out}}}{\text{COD}_{\text{in}}} \right) \times 100\% \quad (27)$$

where COD_{in} (mg L⁻¹) is the influent COD and COD_{out} (mg L⁻¹) is the effluent COD.

The columbic efficiency (CE) was measured as the ratio between the electrons transferred to the anode from the microorganism to the theoretical one that can be achieved if all organic substrates were digested by the microorganisms to generate electrons. Therefore, the CE can be calculated for batch reactor conditions as follows:

$$\mathbf{CE} = \frac{\mathbf{M} \int_0^t \mathbf{I} dt}{\mathbf{n} \mathbf{F} \mathbf{V}_{\text{an}} \Delta \mathbf{COD}} \quad (28)$$

where M stands for the molecular weight of oxygen (32 g L⁻¹), I is the generated current and t is time at maximum power density. n stands for the number of electrons transferred per mole of oxygen, F is Faraday's constant, V_{an} is the volume of anode chamber, and ΔCOD illustrates difference in COD concentration over time.

The MR concentration was determined by measuring a standard curve at a maximum wavelength of 428 nm using UV-Vis spectrophotometry. Therefore, the decolorization efficiency (DE) of MR can be calculated as follows [169]:

$$\mathbf{Decolorization\ efficiency} = \frac{\mathbf{A-B}}{\mathbf{A}} \times \mathbf{100\%} \quad (29)$$

A is the initial absorbance while B is the observed absorbance over time change.

4. RESULTS AND DISCUSSION

4.1. Characterization test

4.1.1. Regression and statistical model analysis for α -MnO₂ NPs

The model analysis to represent linear relationship between two variables is statistical correlation while to fit a best line as well as to estimate one independent variable on the basis of another variable is regression. Therefore, for our experimental work, the regression coefficient values (coefficient $R^2 = 0.9790$) means that 97.90% of both observed and predicted data can be explained by using this model. The predicted R^2 of 0.8452 is in reasonable agreement with the Adjusted R^2 of 0.9496; *i.e.* the difference is less than 0.2. On the other hand, an Adequate Precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. So, the ratio of 16.8861 indicates an adequate signal. As a result, this model can be used to navigate the design space. Therefore, in this study, α -MnO₂ NPs was successfully synthesized using *V. amygdalina* leaf extract as a stabilizing and reducing agent while aqueous KMnO₄ solution was used as a precursor at different experimental conditions. The reducing of permanganate is due to the presence of flavonoid (polyphenolic compound) and alkaloid phytochemicals found on *V. amygdalina* leaf [92,170].

From the UV-Vis spectrophotometer responses obtained by measuring the absorption of the MnO₂ NPs chosen at 320 nm and using the second-degree polynomial mathematical equations in terms of coded factors equations are given as follows:

$$\begin{aligned} \text{Sqrt(Absorbance)} &= +0.8667 + 0.0161A + 0.4222B + 0.0118C + 0.0243D - 0.0196AB \\ &- 0.0607AC + 0.0452AD - 0.0063BC - 0.0375BD + 0.0102CD \\ &- 0.0224A^2 - 0.2706B^2 - 0.0215C^2 + 0.0607D^2 \end{aligned} \quad (30)$$

where A, B, C, and D represents extraction, potassium permanganate ion, pH, and reaction time, respectively. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as “+1” and the low levels are coded as “-1”. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

The evaluation of ANOVA (variance) with this quadratic model could successfully describe all the experimental runs as shown in Table 6. To suggest if the fitted model is relevant (significant), experimental data in terms of Fisher's (F-test), probability (P-value), and

coefficient of variation (R^2), and Lack of Fit are used. According to Liu et al. [150], the p-value is the probability that the given statistical model is the same as or greater magnitude than the actual observed results when the null hypothesis is true. Therefore, smaller p-value of the model corresponds to more significance of the results for all physicochemical parameters. From this works, F-values of the analyzed model term is 33.32 while p-values is < 0.0001 . P-values less than 0.0500 indicate the suggested quadratic model is significant. Non-significant lack of fit is good, and we want the model to fit and much enough to optimize the required physicochemical parameters to synthesis an effective MnO_2 NPs with better yield. A high F-value with low p-value (< 0.001) can show that an optimization strategy is better with the proposed designed model [171]. Terms with significant quadratic model indicate that the proposed model is sufficient and adequate to explain the relationship between the experimented independent variables (factors) under the corresponding responses.

Table 6. ANOVA for absorption response.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2.89	14	0.2066	33.32	< 0.0001	significant
A-Extraction	0.0034	1	0.0034	0.5418	0.4786	
B-Permanganate ion	1.68	1	1.68	270.55	< 0.0001	
C-pH	0.0021	1	0.0021	0.3326	0.5769	
D-Time	0.0075	1	0.0075	1.21	0.2979	
AB	0.0033	1	0.0033	0.5245	0.4855	
AC	0.0372	1	0.0372	5.99	0.0344	
AD	0.0175	1	0.0175	2.83	0.1235	
BC	0.0003	1	0.0003	0.0545	0.8202	
BD	0.0101	1	0.0101	1.63	0.2301	
CD	0.0011	1	0.0011	0.1712	0.6877	
A ²	0.0015	1	0.0015	0.2442	0.6318	
B ²	0.1791	1	0.1791	28.88	0.0003	
C ²	0.0013	1	0.0013	0.2081	0.6580	
D ²	0.0088	1	0.0088	1.42	0.2611	
Residual	0.0620	10	0.0062	-	-	
Lack of Fit	0.0571	7	0.0082	5.04	0.1059	not significant
Pure Error	0.0049	3	0.0016	-	-	-
Cor Total	2.95	24	-	-	-	-

df = degree of freedom

To see the effects of four variables on MnO_2 NPs yield, observing the best insight about the two mutual effects of physicochemical parameters for their responses while keeping the other two variables constant is used to determine their effect responses as shown in the 2D

contour plots and 3D graphs. In addition, to analysis the degree of sensitivity behind each physicochemical parameter, F-values of the model helps to prioritize the dominance of the factor that affects the experimental run. From an experimental planning, the orders of influences on the biosynthesis of MnO_2 NPs are: B-Permanganate ion initial concentration > D-Reaction time > A-Extraction ratio > C-pH. From these order, the initial KMnO_4 concentration has the most positive significant effect while pH has the least effect on the biosynthesis of MnO_2 NPs yield which was clearly supported from the perturbation plot.

Figure 26a indicated that an increase in initial potassium permanganate concentration plays a more significant role for MnO_2 NPs yield. The yield of MnO_2 NPs increases as the precursor concentration increase within the given plant leaf extraction ratios. With increase of permanganate initial concentration, the maximum absorbance by the biosynthesized MnO_2 NPs reaches at $1.5 < \text{absorbance} < 2$, which is clearly shown on the contour plot. Figure 26b illustrates the relationship between permanganate initial concentration and pH. At a higher precursor initial concentration with $5 < \text{pH} < 7$ results the possibility of higher yield MnO_2 NPs formation. Figure 26c revealed that UV-Vis absorption by the biosynthesized MnO_2 NPs yield was better at medium pH and higher reaction time. From these two interactions it is suggested that when pH and reaction time increases, the formed yield is not much effective due to the relative lower electronic absorbance response. The whole analysis indicated that the optimized methodology can help to determine optimized physicochemical parameter which is responsible for stabilized MnO_2 NPs formation. The enhancement of initial permanganate ion concentration and reaction time is a key issue to obtain maximum MnO_2 NPs yield. Generally, the visualization of variation in the physicochemical parameters of bioreduction is coming from the differences in the activities and presence of bioactive biomolecules present in the leaf extract. The presence of such bioactive molecule promotes the crystallization process of metal oxides [172].

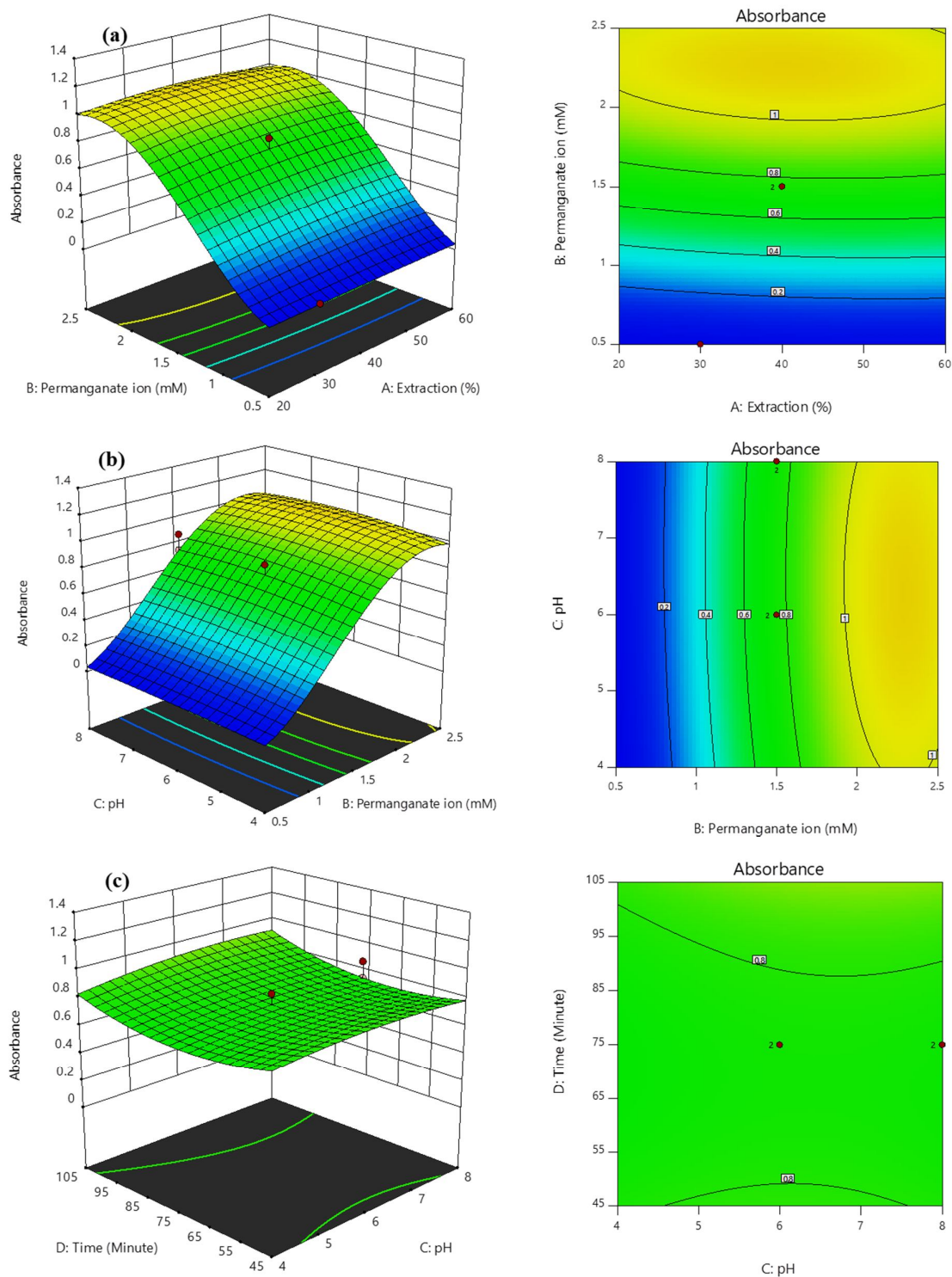


Figure 26. 3D response surface and 2D contour plot for interaction effect of two-parameter on absorption response at fixed values of other parameters, (a) Influence of extraction ratio and initial concentration of permanganate ion on absorbance, (b) Influence of initial concentration of permanganate ion and pH on absorbance, and (c) Influence of pH and reaction time on absorbance.

Optimization of the input variables for all the model study was performed through a desirability function shown in Figure 27. It signifies that how much the optimization conditions specified by Design Expert 11 software are desirable for practical applicability. Desirability value varies within a range of 0.00 (undesirable) to 1.0 (desirable), a value more than 0.5 and closer to 1 is expected for feasibility [173]. Desirability value for all factors were well above 0.5 and hence confirm practical feasibility.

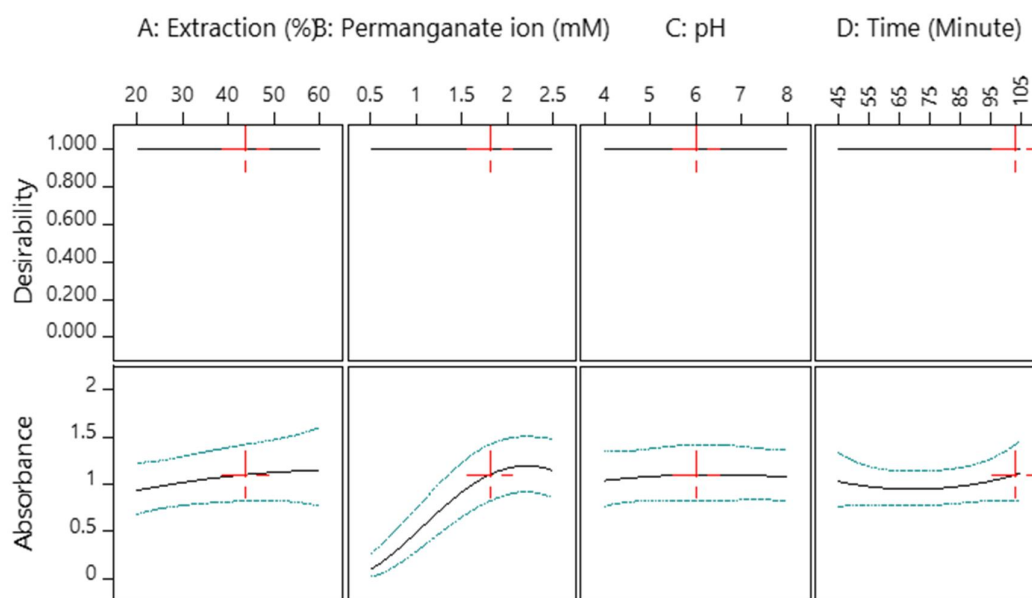


Figure 27. The values of optimum conditions obtained from numerical optimization along with their desirability for stabilized MnO_2 NP.

The experimental results obtained from the defined optimum conditions were done as follows to confirm the negligibility of the error (validation analysis). The validation experimental run was performed according to the optimum production conditions of MnO_2 NPs synthesis. An aqueous solution of initial potassium permanganate concentrations (~ 1.8 mM) was prepared in pH of 6.02. To synthesis the required MnO_2 NPs, a volumetric ratio with $\sim 44\%$ of *V. amygdalina* crude extract was added to the solution as well and stirred at room temperature for ~ 104 minutes reaction time. The precipitate was obtained by centrifuging at 4000 rpm for 15 minutes. Then, the obtained NPs were washed with distilled water and ethanol several times. Some of the washed samples were suspended in 10 mL distilled water for UV-Vis spectrophotometric measurements. The model predicted that the synthesis of MnO_2 NPs yield was 1.095, while, experimental verification was accomplished using the same optimal value and 0.9678 MnO_2 NPs yield was obtained at 320 nm maximum wavelengths from four experimental runs shown in Table 7 with a difference of 0.1272

(12.72 % error), which was lower than 0.2 or 20 % error values. Since 0.2 numerical value is a standard value in Design-Expert 11 software. Therefore, the difference value is lower than the standard error value and this approved that the model is adequate for the optimization of the required physicochemical parameter.

Table 7. Absorbance (at 320 nm) results of the four-experimental set up at optimum condition.

Experiments	Maximum absorbance (at 320 nm)
1	0.8706
2	0.9766
3	1.0649
4	0.9589
Average	0.9678

In aqueous medium MnO₂ NPs appears dark brown in color is due to the surface plasmon vibrations [174]. The biosynthesized NPs show two broad absorption bands ranging between 280 and 350 nm with peak positions at about ~ 285 and ~ 320 nm is the characteristic nature of alkene (double bond) and electron transition from unpaired “n” electron to antibonding “ π^* ” one (i.e., $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ each of which might be the bandgap absorption and transmission spectra of indol adsorption MnO₂ NPs. It should be noted that the absorbance peak of MnO₂ NPs sol might be also changed [61]. The MnO₂ NPs showed absorption maxima at 285 nm which is in good agreement with the literature [151]. Ede et al. [175] have reported the occurrence of maximum absorption peak at $\sim 275 \pm 25$ nm due to dimensional structure difference of the formed NPs. When the aqueous plant (light yellow inset Figure 28a, m) extract was mixed with KMnO₄ solution (purple in color absorption maxima at 310 nm and 530 nm, inset Figure 28a, k), formation of dark brown color that confirms the formation of MnO₂ NPs (Inset Figure 28a, n). In this study, the absence of peak at about 530 nm from KMnO₄ spectra after dispersing *V. amygdalina* extract indicates the complete reduction of Mn(VII) to Mn(IV) and shifting in to lower wavelength of the spectra indicating the presence of small sized MnO₂ suspension which is in good agreement with the previous study [61].

Furthermore, the spectra of crude extract of *V. amygdalina* shown a sharp peak near 320 nm (Figure 28a blue curve, $n \rightarrow \pi^*$ transitions), confirmed the presence and characteristics of alkaloid indole spectra [92]. Indoles are amongst the most important class of heteroaromatics (heterocyclic compound) in organic chemistry, being commonly found in biologically active natural products that easily participates in chemical reactions [176,177].

It could be seen that there is no difference in the absorption peaks position except peak shape, this happening might have two reasons: i) due to adsorption of indol moiety on synthesized MnO₂ NPs ii) the chemical structure difference in *V. amygdalina* leaf extract resulting in a stronger steric hindrance for indole to form bond with oxide surface [178]. A similar mechanism was also observed between *V. amygdalina* leaf extract and TiO₂ (i.e., there maximum absorption was occurred at 400 nm) [90].

The particle size characteristics of the synthesized material was elucidated by direct optical band gap energy (E_g , eV) transition from Tauc's equation [179] given by:

$$(\alpha h\nu)^2 = A(h\nu - E_g) \quad (31)$$

where A is the constant, $h\nu$ is the photon energy, and α is the absorption coefficient. The higher E_g of green synthesized MnO₂ NPs was achieved at 4.65 eV. Which was higher value than the corresponding bulk MnO₂ with allowed direct band gap of 2.7 eV [180]. The increasing E_g with a value of 4.65 eV shown in Figure 28b is attributed to decreasing in particle size, such small particle size helps to improve their electrochemical properties and increase efficiency in electrochemical performance for an effective remediation applications [181,182].

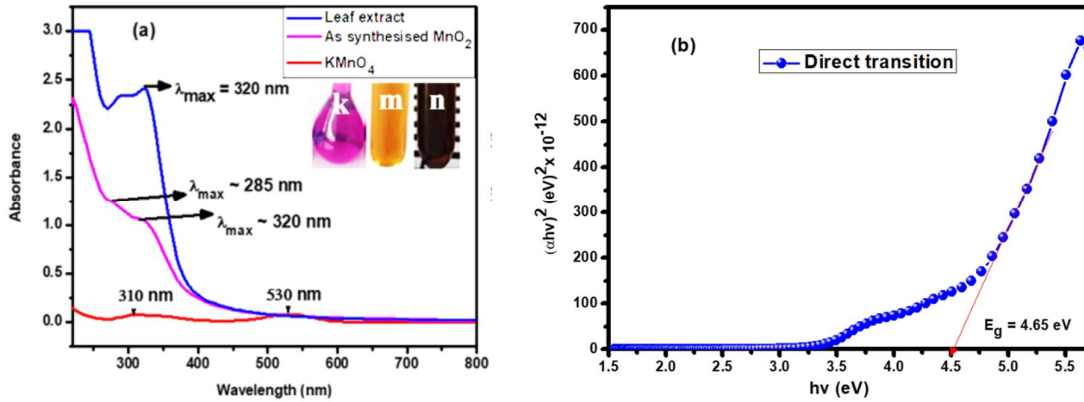


Figure 28. (a) UV-Vis absorption spectra of synthesized MnO₂ NPs [inset: (k) 1.8 mM KMnO₄ solution, (m) plant extract, and (n) synthesized MnO₂ NPs] and (b) Tauc's plot of MnO₂ NPs using direct transition mode.

In order to elucidate the colloidal characterization which supports the polydispersity nature of MnO₂ NPs by the oxidation of *V. amygdalina* leaf extract, the spectrum of optimized reaction mixture was recorded in the 400-600 nm absorption band regions. Due to the formation of water-soluble colloidal MnO₂ solution, one step three electron redox reaction

was takes place between Mn(VII) and *V. amygdalina* leaf extract as a reducing agent. Zaheer [183], suggested that the ligand/or reductant to metal charge transfer transitions observable in the half ultraviolet and near visible regions is the most characteristic behavior for Mn(IV) ion. Band gap coverage with in these regions of the spectrum confirms the formation of colloidal nature of MnO₂ NPs (Figure 29). To support such insight mostly a law described by Rayleigh's was also used for manipulating such solution formation due to the scattering of light. Thus, redrawing the plot of log(absorbance) as a function of log(wavelength) have to be linear to support the formation colloidal MnO₂ NPs. Hence, from the plot, a straight line with a regression coefficient of $R^2 = 0.9855$ followed by negative slope with -4.15 (inset Figure 29) between log(absorbance) and log(wavelength) as derived from Beer's law. Such findings are in good agreement with other literatures [183]. Therefore, it is conclude that the recommended data are consistent with the formation of colloidal MnO₂ NPs during the reduction of KMnO₄ by *V. amygdalina* leaf extract under the optimized experimental conditions.

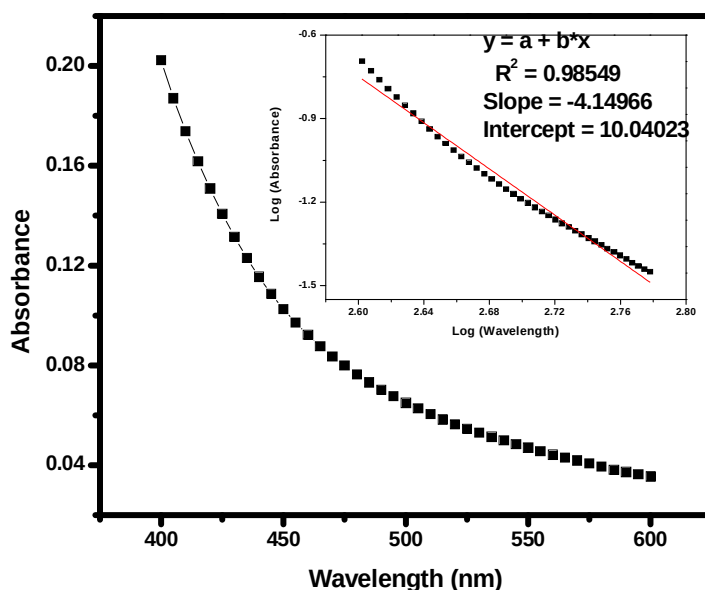


Figure 29. UV-Vis absorption spectra of brown colored MnO₂ NPs as a function of wavelength obtained at optimum experimental conditions. Inset-plot of log (absorbance) versus log (wavelength).

4.1.2. Data analysis for MnO₂/NiO binary composite

The maximal absorption edge that describes composite sizes are shown in Appendix Figure A3 in the wavelength rage between 300 nm-400 nm indicates the bimetallic oxide NPs characteristics from the mixed MnO₂ and NiO [155] NPs formation. The decrease or

increase in band gap which is calculated from each absorption edge using Tauc relation, indicates that the particles size depends on the principal host in which the metal NPs is embedded. But, in most of the cases, E_g decreases with rise in size of the prepared NPs due the red-shift of absorption peak of NPs. The E_g of green α -MnO₂/NiO nanocatalyst is achieved at higher E_g . That is attributed to decreasing in particle size of the synthesized NPs.

The data were analyzed from two factor interaction (2FI) regression model shown in Table 8. The statistical analysis (ANOVA) confirmed that B, C, AB, AC are significant model terms (*i.e.*, their *p*-values are less than 0.05). The nickel ion concentration and reaction time with *p*-value < 0.05 was much effective parameter than plant extract ratio. According to the model, both regression coefficient $R^2 = 0.9873$ and adjusted $R^2 = 0.9494$ confirms that a good correlation is formed between actual and predicted values. An adequate precision measures the signal to noise ratio. So, a ratio greater than 4 is desirable. Therefore, for this work a ratio of 16.8772 indicates the signal is so adequate and thus model is enough to navigate the design space.

Table 8. Analysis of variance for 2FI model.

Source	Sum of Squares	df ^k	Mean Square	F-value	p-value	
Model	1635.39	6	272.56	25.99	0.0375	significant
A-Extract ratio	45.49	1	45.49	4.34	0.1727	-
B-Nickel ion con.	1267.60	1	1267.60	120.88	0.0082	significant
C-Time	420.54	1	420.54	40.10	0.0240	significant
AB	326.74	1	326.74	31.16	0.0306	significant
AC	1088.05	1	1088.05	103.76	0.0095	significant
BC	14.65	1	14.65	1.40	0.3587	-
Residual	20.97	2	10.49	-	-	-
Cor Total	1656.36	8	-	-	-	-
R^2	0.9873	-	-	-	-	-
Adjusted R^2	0.9494	-	-	-	-	-
Adeq Precision	16.8772	-	-	-	-	-

^kDegree of freedom

Effect of operational variables and their interactions are shown in Figure 30. The results marks that the most effective operational variables was nickel ion concentration and it illustrates that the higher nickel ion concentration causes the synthesis of α -MnO₂/NiO nanocatalyst to increase while decreasing the plant extract ratio and increasing the reaction time provides a minor positive effect. The intensity of the absorption band is seen to be a function of the amount of *V. amygdalina* leaf extract used in the reaction and the decrease in the extracts concentration led to an increase in the synthesis of α -MnO₂/NiO nanocatalyst at a unity of desirability.

Design-Expert® Software

Factor Coding: Actual

All Responses

Actual Factors

A: Extract ratio = 75.2877

B: Nickel ion con. = 50.9969

C: Time = 25.2825

Responses

Desirability = 1

Absorption edge (nm) = 376.805

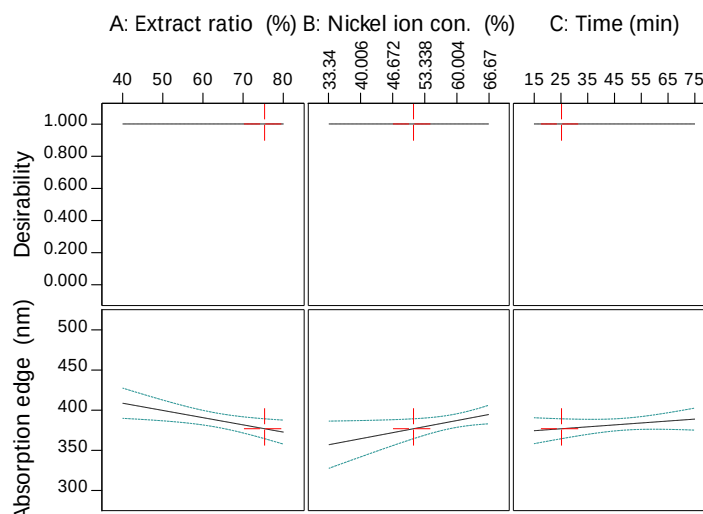


Figure 30. Effect of operational variables on synthesis of α -MnO₂/NiO NPs.

The final influence of each factor by considering the sign and magnitude of each operational variable were summarized in (Equation 32)

$$\text{Absorption edge} = +377.11 + 4.54A + 30.59B - 17.62C - 17.97AB + 32.79AC - 3.07BC \quad (32)$$

where A, B, and C, represents extract ratio, nickel ion concentration, and reaction time, respectively. So, from the equation the largest coefficients were those of the factors AC, B, AB, C, and A, in decreasing order. This shows that the interaction between extract ratio and reaction time imposes the largest impact on the production of MnO₂/NiO bimetallic oxide, followed by nickel ion concentration. The positive sign indicates synergetic effect of the respective term, while the negative term refers to antagonistic effect of the term. Accordingly, the positive value of the coefficient B (+30.59) implies that enhancing the biosynthesis of MnO₂/NiO bimetallic oxide with increasing nickel ion concentration.

Optimization of operational parameters for MnO₂/NiO bimetallic oxide is elucidated using 3D response surface and 2D contour surface plots are shown in Figure 31. Figure 31a and b indicate that the increase in the concentration of nickel ion increases the synthesis of α -MnO₂/NiO nanocatalyst while the increase in reaction time and plant extract ratio did not change the result significantly. The positive and negative residual distribution across the strait line shown in Figure 31c suggests the designed models are so adequate between experimental and predicted values. To elucidate the degree of sensitivity behind each operational parameter, F-values of the model help to prioritize the dominance of the factor that affects the experimental run.

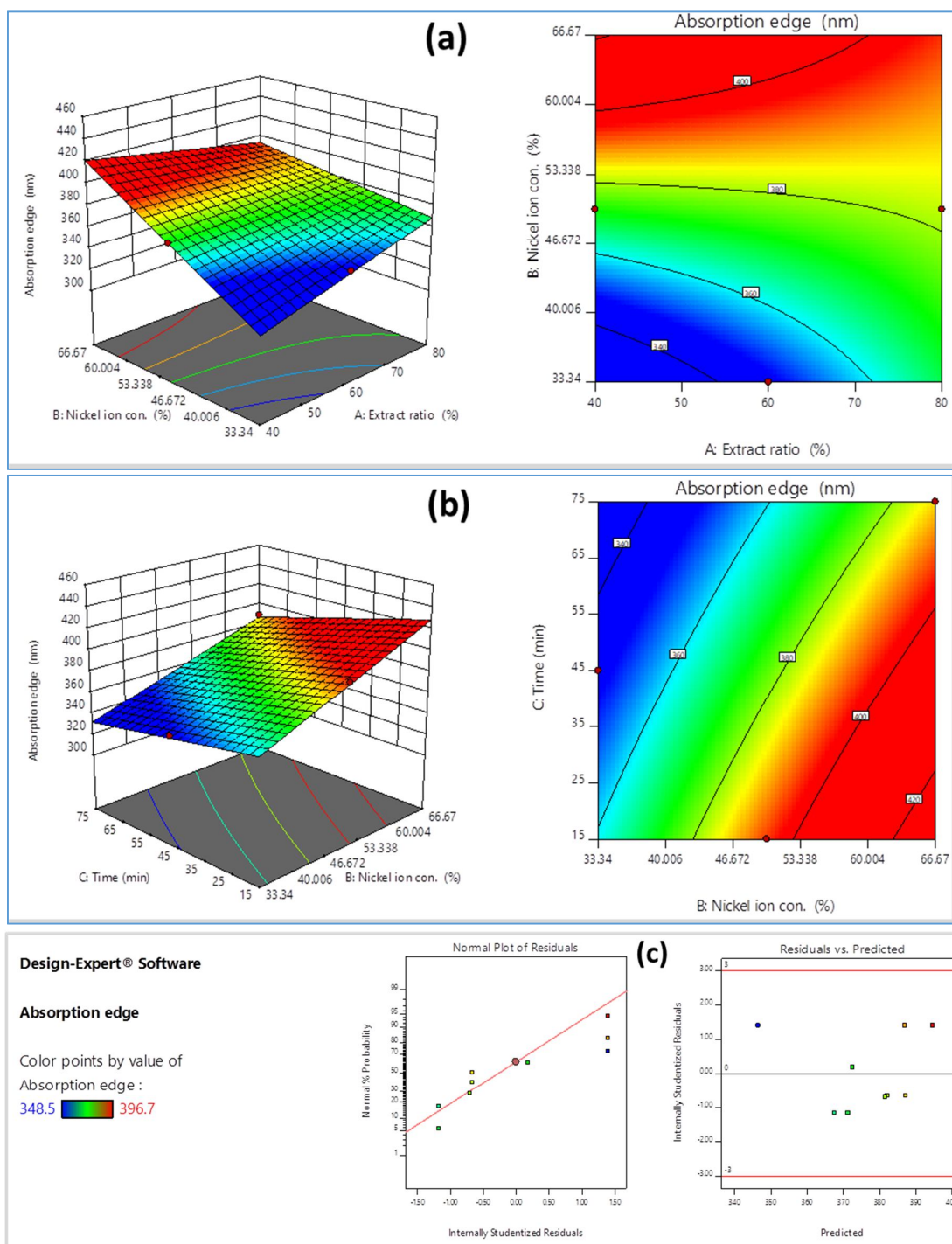


Figure 31. 3D and 2D surface plots and their interaction effect between two-parameters on absorption edge response at fixed values of other operational factors, (a) effects of extract ratio and nickel ion concentration and (b) effects of nickel ion concentration and reaction time, and (c) Residuals of normal plot *versus* predicted on absorption edge.

Keeping the orders of influences based on F-value, the biosynthesis of α -MnO₂/NiO NPs are highly influenced with B-Nickel ion concentration > C-Reaction time > A-Extract ratio. From the order, nickel ion concentration has the dominant and positive significant effect while extract ratio has least effect on α -MnO₂/NiO nanocatalyst yield as supported from perturbation plot Figure 32. Finally, the optimum condition to synthesis α -MnO₂/NiO nanocatalyst was predicted as follows: Nickel ion concentration = 51%, Reaction time = 25.3 minutes, and Extract ratio = 75.3% (v/v) to have a maximal absorption edge of 376.8 nm ($E_g = 3.29$ eV).

Design-Expert® Software

Factor Coding: Actual

Absorption edge (nm)

Actual Factors

A: Extract ratio = 60

B: Nickel ion con. = 50.005

C: Time = 45

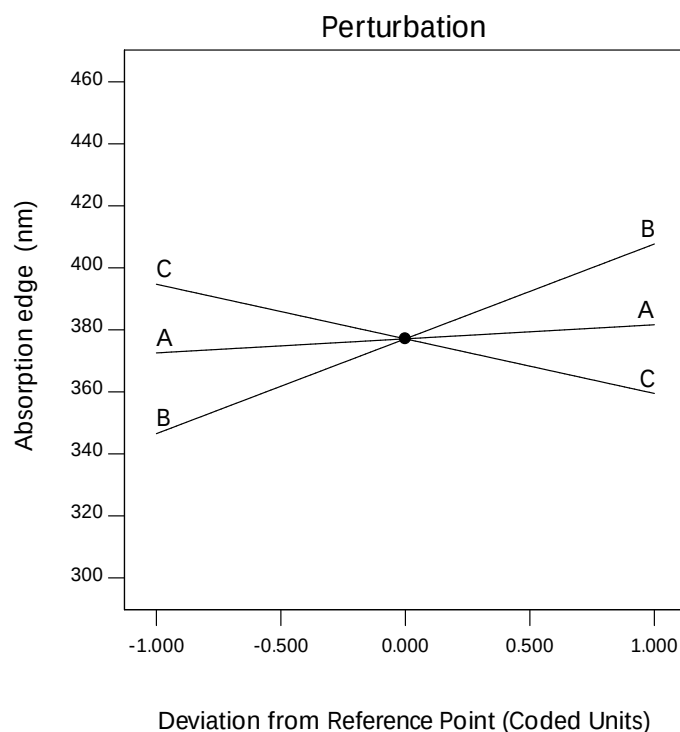


Figure 32. Perturbation plot of MnO₂-NiO NPs at optimized (A) Extract ratio, (B) Nickel ion concentration, and (C) Time actual factors.

The model validation for the biosynthesis of α -MnO₂/NiO nanocatalyst was checked by governing with two set of predicted conditions. The first one is focuses when the predicted value was clearly closer to the observed one, as it could saw from the correlation coefficient (R^2) of 0.9873 (see Figure 33a). This relation suggested the proposed model is effectively developed with effective correlations between all operational variables which are very urgent for α -MnO₂/NiO nanocatalyst production. The second way of confirmation test is proposing an experimental run at optimum conditions which implies that three experimental runs were conducted to prove these validations as shown from Table 9. The validation of

the experimental run was performed by comparing both experimental and predicted α -MnO₂/NiO NPs mean energy band gap values after optimized biosynthesis of α -MnO₂/NiO NPs. Histograms that are shown in Figure 33b predicted that the maximum absorption edge wavelength during the biosynthesis of α -MnO₂/NiO was 376.8 nm (3.29 eV) while experimental verifications confirmed at the same optimal value with E_g of 3.34 eV (370.8 nm) was obtained from triplicate experimental run with a difference of 0.05 (5% error), which was lower than 0.2 or 20% error values. Since 0.2 numerical value is a standard value in Design-Expert 11 software. Therefore, the difference value is lower than the standard value and this approved that the model is adequate for the optimization of operational parameters.

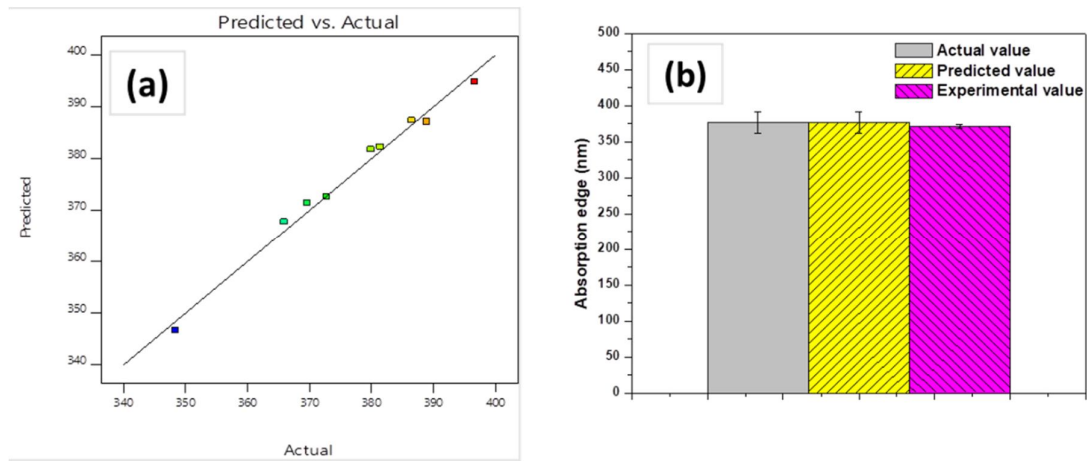


Figure 33. Relationship between predicted, actual, and experimental values of biosynthesized α -MnO₂-NiO mixed metal oxide NPs yield.

Table 9. Optimized experimental results (triplicate results).

Experiments	Absorption edge (nm)
1	368.70
2	370.00
3	373.80
Average	370.80 ($E_g = 3.34$ eV)

Band gap energy from maximum absorption edge wavelength = $1240/\lambda$

4.1.3. Thermogravimetric analysis (TGA)

The thermal stability of α -MnO₂, PANI, α -MnO₂/PANI, and MnO₂/NiO/PANI composite were investigated by TGA (Figure 34). As shown in Figure 34a, the α -MnO₂ has a weight loss of 0.660 mg (5.024%) due to existing water removal from the surface and lattice of the nanostructure before 250 °C at about 146.13 °C. The weight loss of 1.101 mg (8.381%) at

888.16 °C is due to the transformation of α -MnO₂ to Mn₂O₃ [184]. The four major stages of weight loss have been observed on PANI thermal behavior (Figure 34b). The first loss observed at about 37.67 °C is due to the loss of volatile impurities while the second loss obtained at about 205.62 °C was attributed to the loss of dopant and deprotonation of the PANI backbone. At 319.78 °C temperature was due to C=C and C-N chain decomposition of PANI whereas the residual product weight formed after 600 °C at about 692.81 °C may different oxidation products after decomposition [59].

Figure 34c, the α -MnO₂/PANI hybrid composite showed better smoother curve with four minimum weight loss levels, it shows its better stability than pure PANI. The high residual weight loss of α -MnO₂/PANI obtained after 509.59 °C was 6.917 mg (82.502%) which suggests that PANI and α -MnO₂ do not undergo complete oxidation and their composite is highly stable. Figure 34d, α -MnO₂/NiO/PANI material followed similar weight loss pattern to that of α -MnO₂/PANI with four major steps of weight loss. The first two stage weight losses up to 150 °C attributed to the loss of volatile impurities and surface adsorbed water. The weight loss from 150 °C to 500 °C might be assigned to oligomers and other remaining residuals removals [185]. To support this, Ansari et al. [185] have justified that the presence of subsequent weight loss found in PANI and its nanocomposites at higher thermal temperature might be thermo-oxidative decomposition of polymer associated with the degradation of the side chain scission of C-N and of the main chain scission of C-C bond [115]. Finally, the numerical weight loss are summarized in Table 10.

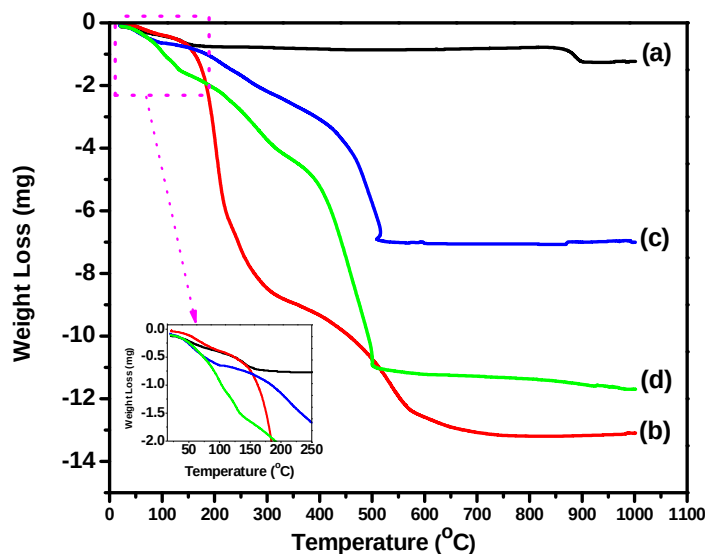


Figure 34. TGA of (a) α -MnO₂, (b) PANI, (c) α -MnO₂/PANI, and (d) α -MnO₂/NiO/PANI samples.

Table 10. Average degradation temperature and their corresponding weight loss analysis between α -MnO₂, PANI, and α -MnO₂/PANI, and α -MnO₂/NiO/PANI.

Sample	Step	Weight Loss		
		Temperature (°C)	TGA (mg)	TGA (%)
α -MnO ₂	1 st	146.13	0.660	5.024
	2 nd	888.16	1.101	8.381
PANI	1 st	37.67	0.072	0.520
	2 nd	205.62	4.393	31.721
	3 rd	319.78	8.732	63.051
	4 th	692.81	13.057	94.281
α -MnO ₂ /PANI	1 st	19.63	0.044	0.5250
	2 nd	225.68	1.433	17.092
	3 rd	493.75	5.440	64.885
	4 th	509.59	6.917	82.502
α -MnO ₂ /NiO/PANI	1 st	84.70	0.644	4.460
	2 nd	198.49	2.088	14.459
	3 rd	473.42	8.855	61.318
	4 th	914.35	11.589	80.251

TGA = Thermogravimetric analysis

4.1.4. Differential thermal analysis (DTA)

Figure 35a attributed that the DTA curve for α -MnO₂ shows two endothermic curves at about 146.13 °C and 888.16 °C that might be due to the loss of physically adsorbed water molecules and phase change from α -MnO₂ to Mn₂O₃ NPs, respectively [186,187]. Figure 35b shows the DTA curve for PANI at 210.46 °C and the endothermic peak may either dehydration or dopant loss of PANI and exothermic peak at about 524.31 °C which is due to PANI chain decomposition (oxidation products) followed by heat loss [188]. The domination of exothermic peak at about 503.44 °C, is due to the high cooling rate capacity of the α -MnO₂/PANI composite with smooth weight loss after 600 °C (see Figure 35c). Decreasing of endothermic peak on the composite than PANI and absence of any other band above 600 °C indicated that hybrid composite is more stable. As a support, a similar fashion have been done between PANI and mesoporous MnO₂ by using H₂SO₄ as a dopant [184]. Finally, the curve shown on Figure 35d, peak at 467.70°C is for α -MnO₂/NiO/PANI. This revealed that α -MnO₂ and α -MnO₂/NiO has a clear interaction with PANI and increases stabilization by peak disappearance of α -MnO₂ near 900 °C. The lower temperature peak existence on both composites than pure PANI due to the fast loss of different products from the polymer decomposition [189]. The summarized peak analysis steps are given in Table 11.

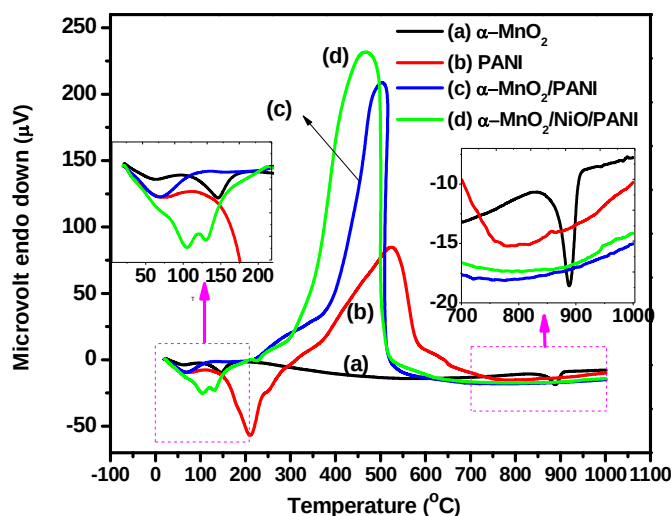


Figure 35. DTA of (a) α -MnO₂, (b) PANI, (c) α -MnO₂/PANI, and (d) α -MnO₂/NiO/PANI samples.

Table 11. DTA peak analysis results for α -MnO₂, PANI, α -MnO₂/PANI, and α -MnO₂/NiO/PANI.

DTA peak	α -MnO ₂		PANI		α -MnO ₂ /PANI		α -MnO ₂ /NiO/PANI	
Step	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
Temperature (°C)	146.13	888.16	210.46	524.31	68.25	503.44	105.12	467.70
DTA (μV)	-9.68	-18.57	-56.90	84.79	-9.36	208.79	-25.30	231.86

4.1.5. Differential scanning calorimetry (DSC) analysis

The DSC thermogram of the biosynthesized MnO₂ NPs is given in Figure 36. For the DSC curve, an endothermic peak was observed in the temperature range between 50 °C and 100 °C which corresponds to the loss of surface absorbed water. In the temperature range of 200-325 °C, two intensive exothermic peaks were observed at average temperature of 255.24 °C and 289.32 °C, due to the fast conversion of hydroxides from MnOOH to two possible manganese oxides, such as MnO₂ and Mn₂O₃, respectively, and further lower heat flow intensities from the thermogram above 325 °C might be the removal of organic moieties from the *V. amygdalina* leaf extracts. Correspondingly, a similar and two exothermic peaks based study were investigated using TGA-DSC analysis from Mn(CH₃COO)₂·4H₂O and (NH₄)₂C₂O₄·H₂O mixtures using air-calcination (oxidation decomposition method) of the MnC₂O₄ precursor at different temperatures followed by acid-treatment in 2M H₂SO₄ solution [190]. Aghazadeh et al. [191] have also reported the three step weight losses from the conversion of hydroxide into the oxide products in the temperature ranges of 25-150 °C,

150-300 °C, and 300-500 °C. The first weight loss justified adsorbed H₂O molecules removal from the hydroxide deposited or intercalated in its structure. The second weight loss can be attributed to the decomposition and removal of cetyl trimethylammonium bromide along with the conversion from MnOOH to MnO₂. The last weight loss is corresponding to conversion from MnO₂ to Mn₂O₃. In related works, the weight loss at the temperature range of 300-500 °C could be also related to the removal of intercalated anions, *i.e.*, chloride ions coming from MnCl₂ precursor [191].

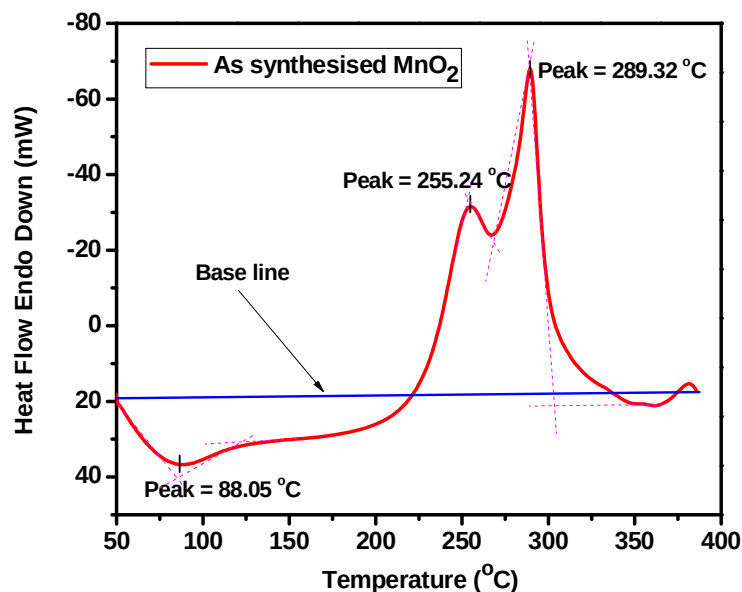


Figure 36. DSC thermogram of as biosynthesized MnO₂ NPs.

After TGA and DTA analysis, biosynthesized MnO₂ NPs was calcined at temperature of 450 °C for 2 and 3 hours. According to literature report 2 and 3 hours calcination time were selected as the most selective time to synthesis thermally stable manganese oxide catalyst in the temperature range of 400 to 500 °C [192–194]. The structural and crystallinity changes during the heating process were further studied by DSC analysis in the presence of nitrogen atmosphere and the results are presented in Figure 37. From the thermogram MnO₂ precursor after 3 hour calcination time (Figure 37b) has better crystalline structure than 2 hour calcination temperature (Figure 37a), due to having lower endothermic heat flow (*i.e.*, sample only needs small absorption energy to melt the precursor). This statement is exactly supported from XRD spectral analysis. The presence of endothermic peaks from both curve indicated the change of phase [195]. Many reports supported that stabilized MnO₂ NPs could be obtained at about 450 °C calcination temperature and this was proved by TGA [191,196–

198]. Occurrence of endothermic peak at a temperature of 181.13 °C and 186.09 °C corresponds to the formation of Mn_2O_3 NMs. The literature justified that the average melting temperature (glass transition (T_g) = 250 °C) was occurred in the temperature range of 150 °C to 300 °C during the thermal decomposition of MnO_2 to Mn_2O_3 under N_2 gas [196].

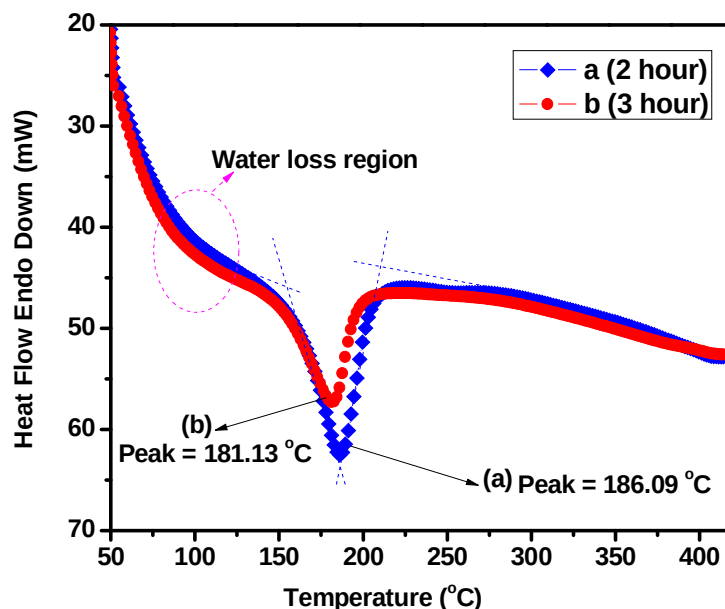


Figure 37. DSC thermogram of calcined $\alpha\text{-MnO}_2$ NPs precursor in N_2 atmosphere (a) 2 hour calcination and (b) 3 hour calcination.

The thermal effect on phase behavior of $\alpha\text{-MnO}_2/\text{NiO}$ bimetallic oxides shown in Figure 38 stated that, endothermic peak occurring at a temperature of 181.13 °C, described the average melting temperature of $\alpha\text{-MnO}_2$ during its the thermal decomposition into Mn_2O_3 under N_2 gas (Figure 38a). The endothermic peak at about 133.08 °C corresponds to the evaporation of physically adsorbed water [199] while ~ 206.40 °C might be the reduction of NiO after melting to Ni under anaerobic condition (Figure 38b) [200]. The reduction of intense endothermic peaks compared to each pure component composed from $\alpha\text{-MnO}_2$ and NiO at about 182.86 °C showed in Figure 40c elucidated that the clear integration between $\alpha\text{-MnO}_2$ and NiO to form $\alpha\text{-MnO}_2/\text{NiO}$ nanocatalyst. Meanwhile, endothermic with intense peak formation at about 136.41 °C is the clear integration between $\alpha\text{-MnO}_2$ and NiO with loss of surface absorbed water found on the mixed oxide nanohybrids.

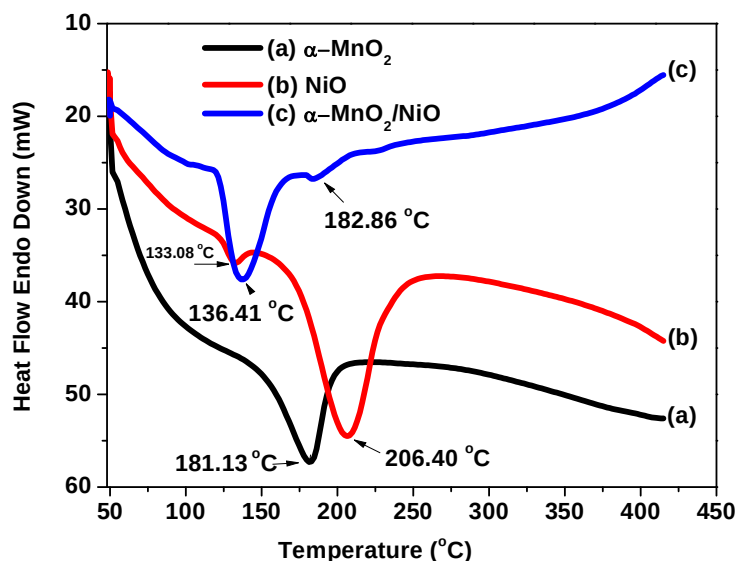


Figure 38. DSC thermogram of (a) α -MnO₂, (b) NiO, and (c) α -MnO₂/NiO.

The thermal effect on phase behavior of α -MnO₂/PANI was done by DSC analysis and shown in Figure 39. Occurrence of endothermic peak at a temperature of 181.13 °C indicated the glass transition (T_g) temperature and its phase change into Mn₂O₃ under N₂ (Figure 39a). The thermographs of PANI show a broad endothermic peak at 116.37 °C, which attributes to the T_g (see Figure 39b). It is reported that, depending up on the terms of synthesis conditions PANI might be extended and occurred near 100 °C [201]. The falling of broad endothermic peaks on each pure component composed from α -MnO₂ and PANI showed the clear integration of α -MnO₂ in PANI matrix to form a new and stable nanocomposite formation with loss of surface absorbed water occurrence at about 83.41 °C while an exothermic temperature after 376.61 °C shows the beginning of decomposition of PANI chain degradation (Figure 39c). The falling of broad endothermic peak on pure α -MnO₂ and PANI components showed the clear integration of α -MnO₂ and α -MnO₂/NiO in conducting PANI matrix to form new and stable nanocomposite formations as shown in Figure 39d.

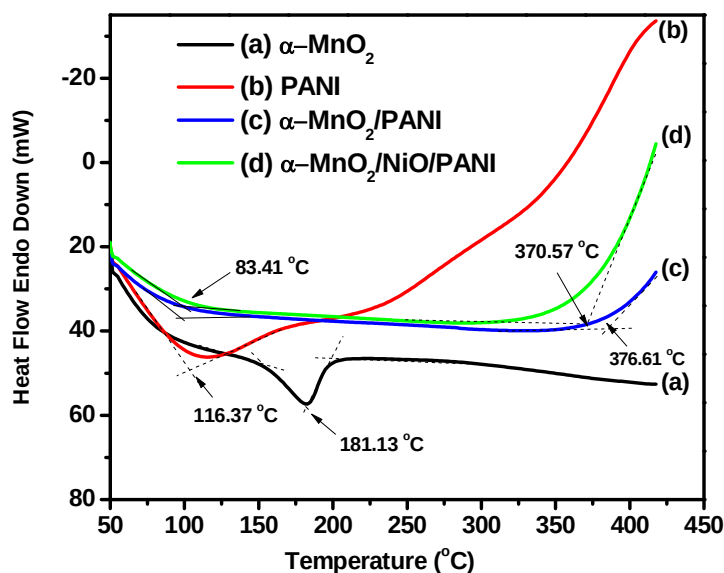


Figure 39. DSC analysis of (a) α -MnO₂, (b) PANI, (c) α -MnO₂/PANI, and (d) α -MnO₂/NiO/PANI samples.

4.1.6. X-ray diffraction (XRD) analysis

Figure 40 shows the XRD patterns of biosynthesized NPs samples using *V. amygdalina* leaf extract. According to the XRD patterns, the crystallinity of the synthesized product was further improved by calcination at 450 °C by extending calcined time from 2 hour to 3 hour, and this behavior is consistent and in good agreement with γ -MnOOH synthesis and its conversion to α -MnO₂ [187] and β -MnO₂ [202]. Since calcination at higher temperature can increase the active surface sites of the synthesized NPs, *i.e.*, increment in degree of porosity on the material [203]. The as synthesized powders which was dried overnight at 80 °C with an average crystallite size of 18.47 nm (Figure 40a) exhibit peaks that can be easily indexed to γ -MnOOH (manganite) of monoclinic phase with cell parameters contains: $a = 5.30400$ Å, $b = 5.27700$ Å, $c = 5.30400$ Å, and $\beta = 114.380^\circ$ (Crystallography Open Database-CryOD, Entry # 96-900-9775 [space group: $P121/c1$ (14)]). After 450 °C thermal treatment with varied calcined time, the crystalline structure of the sample progressively evolves towards α -MnO₂ with pure tetragonal phase and lattice constants (cell parameters) $a = 9.78760$ and Å $c = 2.86500$ Å (CryOD, Entry # 96-151-4117 [space group: $I4/m$ (87)]). XRD patterns of NPs synthesized using the *V. amygdalina* leaf extract exhibit a high intensity peak at 12.479° (Figure 40c), which can be indexed as the (110) plane followed in intensity by the peaks for the plane at 400 (Figure 40b). It is important to note that regardless of the natural extract used for the synthesis, the sample calcined at 450 °C for 3 hour exhibit

an increase in the peak intensity and a decrease in peak broadening when compared to the same samples calcined at 450 °C for 2 hour. Careful comparison of the relative intensities in Figure 40c shows that a highly crystalline structures as evident from the dominant and sharp diffraction peaks of XRD patterns of the α -MnO₂ phase which are indexed at (110), (220), and (211) plane.

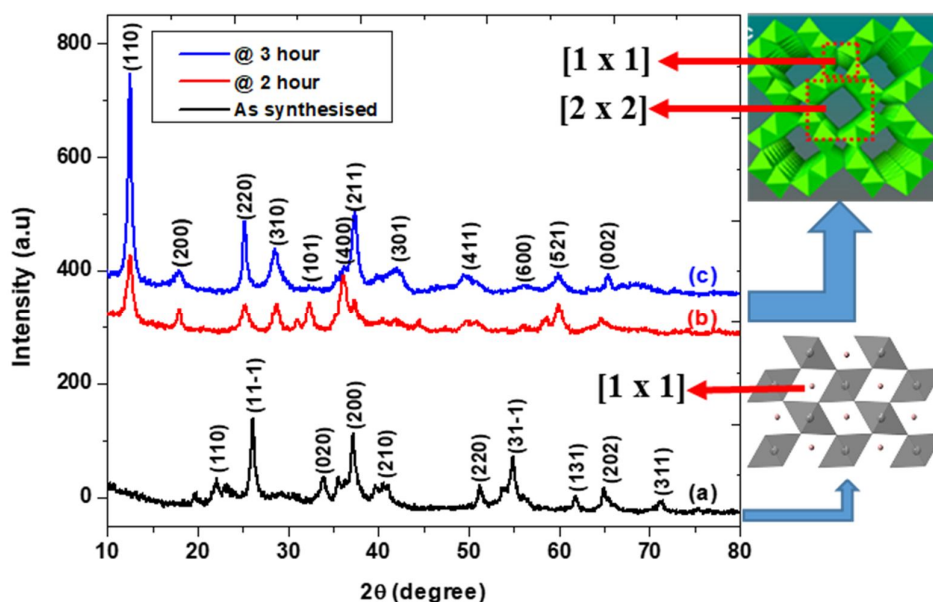


Figure 40. XRD pattern of as synthesized sample (γ -MnOOH) (a), α -MnO₂ NPs calcinated at 450 °C for 2 hours (b), and α -MnO₂ NPs calcinated at 450 °C for 3 hours (c).

From other report, the presence of well-defined XRD peaks indicated the dominance of crystalline structure nature of the synthesized materials [64]. Thus, from Debye-Scherrer relation [204], the individual and average crystallite size of the as synthesized γ -MnOOH and calcined α -MnO₂ NPs are summarized in Table 12.

Table 12. The calculated crystallite size of as synthesized γ -MnOOH and α -MnO₂ NPs (calcined at 450 °C).

Sample type	d-spacing (nm)	2 θ (degree)	FWHM (degree)	Miller indices (hkl)	Crystallite size (nm)
As synthesized γ -MnOOH	0.34085	26.036	0.4009	1 1 -1	21.25
	0.22593	37.155	0.5082	2 0 0	17.23
	0.16712	54.806	0.5516	3 1 -1	16.95
					Average = 18.47
α -MnO ₂ (2 hour calcination)	0.69569	12.971	0.3757	1 1 0	22.24
	0.24623	35.917	0.3811	4 0 0	22.90
	0.15347	59.920	0.6100	5 2 1	15.71
					Average = 20.28
α -MnO ₂ (3 hour calcination)	0.69792	12.479	0.3875	1 1 0	21.55
	0.35008	25.228	0.4050	2 2 0	21.00
	0.23830	37.218	0.4453	2 1 1	19.67
					Average = 20.74

XRD patterns of optimized biosynthesized α -MnO₂/NiO NPs after heat treatment at 450 °C are shown in Figure 41a. Compared to the XRD pattern of pure α -MnO₂ (see Figure 41a (i)), there is an additional peak at $2\theta = 28.420^\circ$, 37.270° , 43.310° , and 62.880° in the diffractograms shown in Figure 41a (ii-iv). Especially, the peak attributed at 28.420° with (310) plane is for α -MnO₂ and the intensity of the peak with peak position shift to the higher 2θ value. As nickel ion content increased, the XRD peaks shift slightly toward higher 2θ values. This effect was due to the fact that Ni²⁺ (69 pm) [205] substitute Mn⁴⁺, which has larger ionic radius than that of Mn⁴⁺ (53 pm) [206]. The substitution of Mn⁴⁺ with Ni²⁺ resulted in a decrease in d-spacing of the cell structure and its values are determined from Bragg's law (see Table 13) [207], which may confirm the incorporation of Ni²⁺ with α -MnO₂ crystal lattice to form α -MnO₂/NiO hybrid.

The XRD pattern of pure α -MnO₂ showed three intense peaks found at 2θ of 12.479° , 25.228° , and 37.218° , which are attributed to the diffraction planes at (110), (220), and (211), respectively (Figure 41b (i)). The represented sample was indexed with the standard XRD pattern of α -MnO₂ (CryOD, Entry # 96-151-4117). The XRD pattern of NiO exhibits three main sharp peaks at 2θ of 37.270° , 43.310° , and 62.880° which are indexed to a

diffraction planes at (111), (200), and (220), respectively, due to the characteristic chain of NiO (Figure 41b (ii)). The highly crystalline NiO NPs displayed sharp and intense diffraction peaks that perfectly matched well with the standard XRD pattern of NiO (CryOD, Entry # 96-432-9324) from Match3 phase identification software and in good agreement with the literature [155]. From this increases by peak intensity on NiO, one can suggest that the crystallite size is larger as supported from Table 13. Figure 41b (iii) shows the XRD pattern of the α -MnO₂/NiO nanohybrids. The peaks at 28.420°, 36.250°, and 40.620° represent the mixed nanocatalyst (CryOD, Entry # 96-403-1181). It can be seen that the diffraction peaks of this nanocatalyst confirmation is evidenced by the standard XRD patterns from α -MnO₂ and NiO due to their clear mixtures. This indicated that α -MnO₂-NiO mixed metal oxide nanocatalyst has successfully fabricated *via V. amygdalina* leaf extract assisted method.

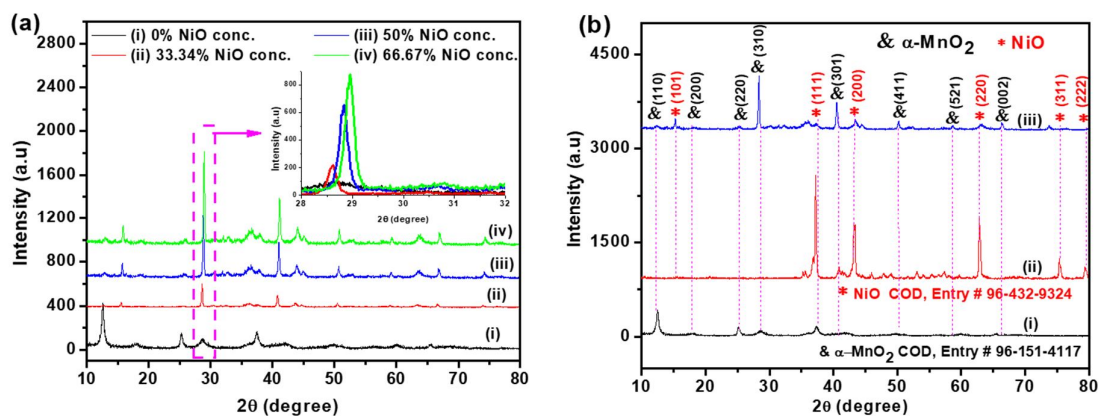


Figure 41. (a) X-ray diffraction patterns of α -MnO₂-NiO bimetallic oxide NPs for different nickel ion concentrations (i-iv) and inset: shift of peak position of α -MnO₂/NiO along (310) plane; (b) XRD patterns of (i) α -MnO₂, (ii) NiO, and (iii) α -MnO₂/NiO NPs after heating at 450 °C.

Table 13. XRD data used for calculation of interlayer spacing, average crystallite size of α -MnO₂, NiO, and α -MnO₂/NiO nanocatalyst.

Sample type	d-spacing (nm)	2 θ (degree)	FWHM (degree)	Miller indices (h k l)	Crystallite size (nm)
α -MnO ₂	0.69792	12.479	0.3875	1 1 0	21.55
	0.35008	25.228	0.4050	2 2 0	21.00
	0.23830	37.218	0.4453	2 1 1	19.67
	Average = 20.74				
NiO	0.24125	37.270	0.2000	1 1 1	43.77
	0.20893	43.310	0.2000	2 0 0	44.62
	0.14774	62.880	0.2000	2 2 0	48.61
	Average = 45.67				
α -MnO ₂ /NiO	0.31493	28.420	0.2000	1 0 1	42.78
	0.24373	36.250	0.4000	0 1 2	21.82
	0.22220	40.620	0.2800	2 0 0	31.59
	Average = 32.06				

The crystalline structure of α -MnO₂/PANI hybrid composite was performed by XRD test as shown in Figure 42. The XRD pattern of pure α -MnO₂ is shown in Figure 42a with three intense peaks found at 2 θ of 12.479°, 25.228°, and 37.218° with lattice plane of (110), (220), and (211), respectively. The XRD pattern of PANI exhibits two main broad peaks with 2 θ of 20.494° and 25.413°, which are indexed to a diffraction planes at (100) and (110), respectively, due to the characteristic chain of PANI (Figure 42e) [208].

To see the structural effect on α -MnO₂/PANI hybrid composite, different mass fraction of α -MnO₂ NPs was tested and shown in Figure 42b-d. From this, as the mass fraction of α -MnO₂ NPs decreases its crystallinity was affected and its peaks become broad indicated a clear combination of amorphous PANI structure. The long and sharp intense peaks decreased very rapidly as shown in Figure 42c due to the fast interaction between PANI and α -MnO₂ leading to PANI formation on α -MnO₂ surface, as reported elsewhere [209]. Such interaction eliminate the crystallinity structure of α -MnO₂, and shows the transformation of the crystalline α -MnO₂ into the amorphous phase of PANI, which is in good agreement with the patterns reported for α -MnO₂ synthesized using a similar process [208]. When the amount of MnO₂ NPs decreases the diffraction peaks of the composites become broad and their peak positions are almost similar with pure PANI in all planes except diffraction angle at 21.151° with indexed to (100) (Figure 42d). So, this confirms that successful interaction between α -MnO₂ and PANI. The calculated crystallite sizes of all samples are shown in Table 14.

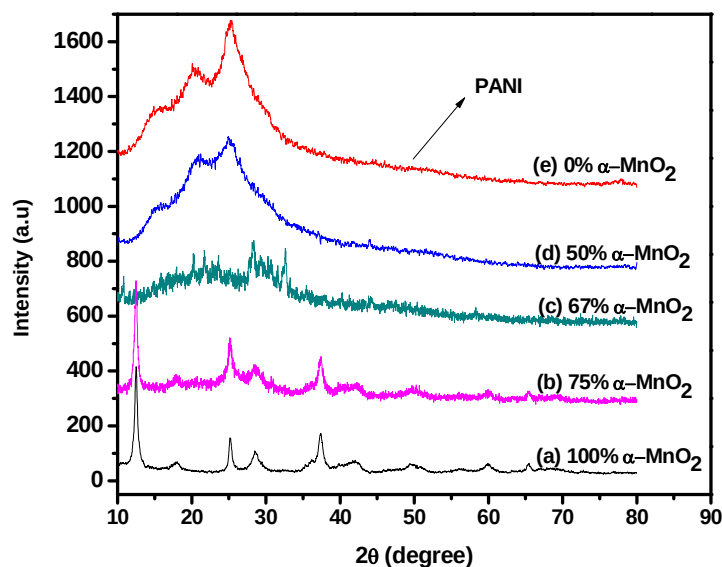


Figure 42. XRD patterns of pure α -MnO₂ NPs (a), α -MnO₂/PANI nanocomposites of different mass ratio (b-d), and that of pure PANI (e).

Table 14. Average crystallite size of α -MnO₂, PANI, and of α -MnO₂/PANI nanocomposites at different mass ratios.

Sample type	Amount of MnO ₂ NPs (wt% to v)	Peak position	2 θ (degree)	FWHM (degree)	Crystallite size (nm)
α -MnO ₂	100	1	12.479	0.3875	21.55
		2	25.228	0.4050	21.00
		3	37.218	0.4453	19.67
		Average = 20.74			
α -MnO ₂ /PANI	75	1	12.473	0.4580	18.23
		2	25.320	0.4771	17.83
		3	37.381	0.6449	13.59
		Average = 16.55			
α -MnO ₂ /PANI	67	1	21.823	2.594	3.26
		2	28.194	6.778	1.26
		Average = 2.26			
		1	21.151	1.545	12.48
α -MnO ₂ /PANI	50	2	25.413	14.831	0.54
		Average = 6.51			
PANI	0	1	20.494	2.245	3.56
		2	25.413	10.024	0.79
		Average = 2.18			

The XRD patterns of α -MnO₂/NiO/PANI nanocomposite is similar to that of α -MnO₂/PANI nanocomposite, as shown in Figure 43e. The peaks due to PANI at $2\theta = 20.345^\circ$ and 25.684° , respectively, shifted from $2\theta = 20.494^\circ$ and 25.413° , which may be attributed to the interaction of PANI with α -MnO₂ in-built on NiO multilayer microstructure. The four peaks observed in the α -MnO₂/NiO/PANI nanocomposite at $2\theta = 38.322^\circ$, 43.897° , 64.378° , and

77.583°, may be indexed to the (111), (200), (220), and (311) planes, respectively, due to the reflection of NiO integration on PANI matrix with a slight shift compared to those in the α -MnO₂/PANI nanocomposite, indicating the successful preparation of α -MnO₂/NiO/PANI nanocomposite [210]. The overall crystallite size and d-spacing comparison of α -MnO₂/NiO/PANI nanocomposite with other is summarized in Table 15.

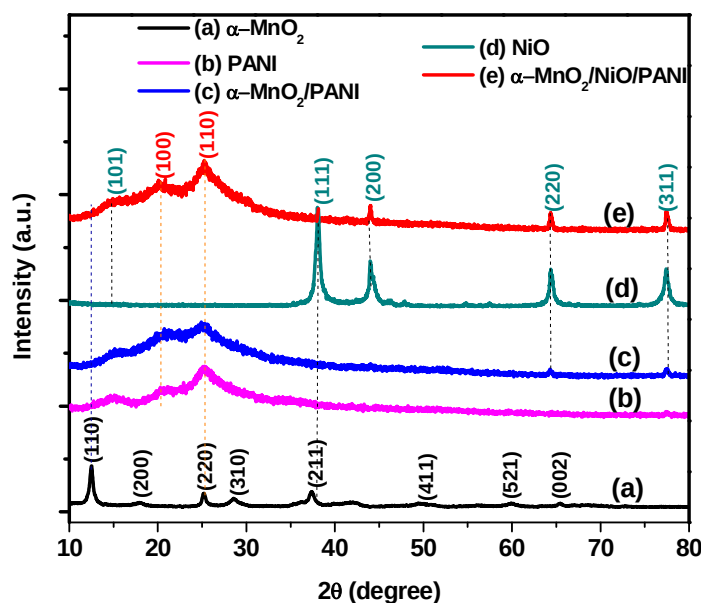


Figure 43. XRD patterns of α -MnO₂ (a), PANI (b), α -MnO₂/PANI nanocomposite (c), NiO (d), and α -MnO₂/NiO/PANI nanocomposite (e).

Table 15. XRD data used for calculation of interlayer spacing, average crystalline size for α -MnO₂, PANI, α -MnO₂/PANI, and α -MnO₂/NiO/PANI NMs.

Sample type	d-spacing (nm)	2θ (degree)	FWHM (degree)	Miller indices (h k l)	Crystallite size (nm)
α -MnO ₂	0.69792	12.479	0.3875	1 1 0	21.55
	0.35008	25.228	0.4050	2 2 0	21.00
	0.23830	37.218	0.4453	2 1 1	19.67
					Average = 20.74
PANI	0.43275	20.494	2.245	1 0 0	3.56
	0.38851	25.413	10.024	1 1 0	0.79
					Average = 2.18
α -MnO ₂ /PANI	0.41971	21.151	1.545	1 0 0	12.48
	0.35021	25.413	14.831	1 1 0	0.54
					Average = 6.51
NiO	0.24125	37.270	0.2000	1 1 1	43.77
	0.20893	43.310	0.2000	2 0 0	44.62
	0.14774	62.880	0.2000	2 2 0	48.61
					Average = 45.67
α -MnO ₂ /NiO/PANI	0.62286	20.345	2.835	1 0 0	2.95
	0.34657	25.684	11.775	1 1 0	0.72
					Average = 1.84

4.1.7. Ultraviolet-Visible (UV-Vis) absorption analysis

The fabrication of optimized α -MnO₂/NiO nanocatalyst was evidenced from UV-Vis absorption spectroscopy and their spectra are given in Figure 44. UV-Vis absorption spectra exhibited absorption band between 250 nm and 350 nm. At about 285 nm showed the characteristics band for optimized α -MnO₂ NPs (Figure 44b). For comparison, NiO NPs has been synthesized using *V. amygdalina* leaf extract via green method and its maximum absorption edge was observed at wavelength of 291 nm as shown at Figure 44a. The absorption spectrum of the NiO NPs shows a broadening spectrum at the absorption edge of 260 nm which is in good agreement with the literature [211]. Moreover, its maximum absorption is occurred at different wavelength due to different precursor type [211]. The lower in wavelength shift and increased in band gap of the synthesized NiO NMs than that of the bulk indicated decrease in particle size due to the existence of quantum confinement effect [155]. The UV-Vis absorbance spectrum of biosynthesized α -MnO₂/NiO nanocatalyst using the *V. amygdalina* leaf extract (Figure 44c) showed the significant changes in the absorbance maxima at about 259 nm towards lower in wavelength shift than α -MnO₂ NPs. The slight shift towards lower wavelength attributed to smaller size formation of α -MnO₂-NiO bimetallic oxide NPs. The lower deviations during the time of visible scan revealed that the better stability of bimetallic oxide NPs.

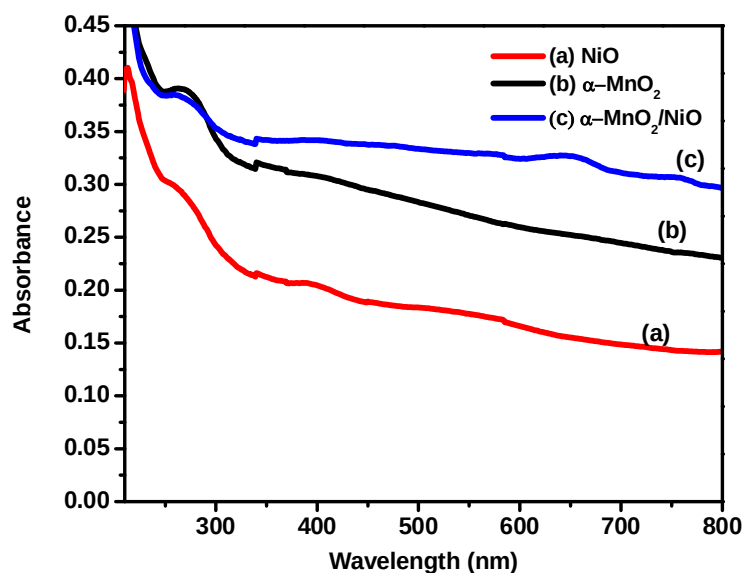


Figure 44. UV-Vis absorption spectra of NiO (a), α -MnO₂ (b), and α -MnO₂/NiO (c).

The incorporation through insertion of biosynthesized α -MnO₂ NPs in PANI conducting polymer matrix to form α -MnO₂/PANI composite were also evidenced from UV-Vis

absorption spectroscopy and their spectra are given in Figure 45. The optimized α -MnO₂ exhibited with absorption band at about 285 nm is due to charge transfer from O_{2p} valance band to Mn_{3d} conduction band as shown in Figure 45a. The absorption bands at 267 nm are related to the excitation of electrons from amine segments in the benzenoid $-(C_6H_4)-$ rings while band near 405 nm formed in the range of 400-450 nm corresponds to transition from $\pi \rightarrow$ polaron (see Figure 45b). The absorption bands at 740 nm are related to the excitation of electrons from imine segments in the quinoid ring of PANI. It is important that in 740 nm, some segments of the PANI backbone are distorted locally. This distortion creates a localized molecular exciton with the electron and a hole on a quinoid moiety and on the two neighboring benzenoid moieties, respectively [156]. This could be simplified as electron excitation between the highest occupied molecular orbital of the benzene ring (n) and the lowest unoccupied molecular orbital (π^*) of the quinoid ring that provide an adsorption band on 740 nm. From such evidence (formed polaron) it might be deduced that the synthesized PANI is in emeraldine form [212]. In α -MnO₂/PANI structure shown in Figure 45c indicated a lower wavelength shift which was observed for the band from 405 nm absorption maxima of PANI to 340 nm absorption edge composite, which was ascribed to the $n-\pi^*$ transition. This shifting may be related to the possible interaction of the α -MnO₂ with the PANI ring, leading to the formation of a polymer nanocomposite. In the case of α -MnO₂/NiO/PANI ternary nanocomposite shown in Figure 45d, a PANI wavelength shift from 405 nm to $\sim$ 291 nm and $\sim$ 343 nm with increasing in intensity than α -MnO₂, PANI, and α -MnO₂/PANI related the loading of both NiO and α -MnO₂ NPs in benzenoid moieties of PANI conducting polymer. The interactions were in good agreement with other report found in the literature [156]. So, the significance in wavelength change in all nanocomposite supports their best in electrical conductivity due to the polaron movement across the extended conjugation [210].

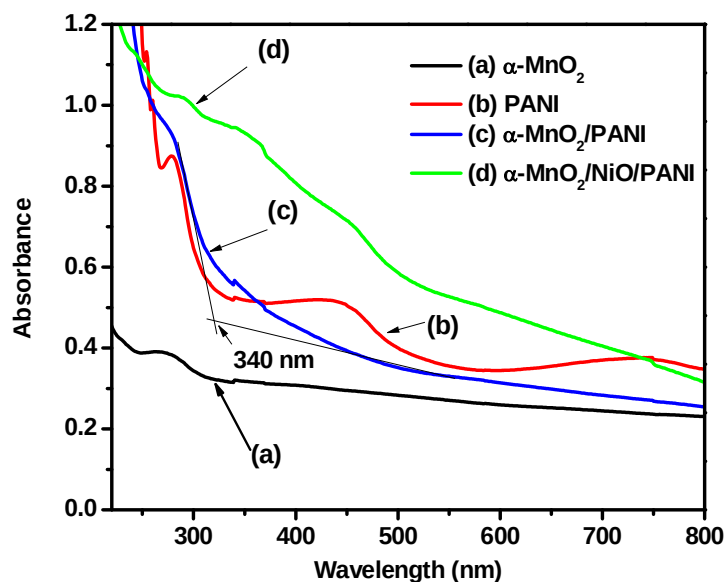


Figure 45. UV-Vis absorption spectra of α -MnO₂ (a), PANI (b), α -MnO₂/PANI nanocomposite (c), and α -MnO₂/NiO/PANI nanocomposite (d).

4.1.8. Fourier transform infrared (FTIR) spectroscopy analysis

FTIR is a spectroscopy that used to detect both inorganic and organic species containing a sample with low content due to its high sensitivity [213]. Figure 46a shows the FTIR spectra corresponding to the as synthesized sample prepared with *V. amygdalina* leaf extract, while the spectra shown in Figure 46b and c corresponding to MnO₂ NPs calcined at 450 °C for 2 and 3 hours, respectively. The occurrence of broad and low in intensity peak from the as synthesized sample at about 3422 cm⁻¹ and 3304 cm⁻¹ might be assigned as –OH stretching and H-O-H bending vibrations of bounded water molecules that are adsorbed on the sample, respectively [93]. The peak at about 2925 cm⁻¹ and 2845 cm⁻¹ characterized the C-H stretching absorption band due to the presence of alkene/alkyl groups from secondary phytochemicals organic compounds found in *V. amygdalina* [91,92]. The peak observed around 1643 cm⁻¹ showed water molecule bending vibration mode [214]. An intense and sharp peak observed at about 1563 cm⁻¹ indicated C=O stretching [204]. The absorption band formed at about 1401 cm⁻¹ and 1079 cm⁻¹ indicated C=C unsaturation and C-O stretching, respectively [215]. Finally, the peak observed at about 910 cm⁻¹ indicates the surface –OH groups of Mn-OH for colloidal MnO₂ NPs while the band between 600 cm⁻¹ and 475 cm⁻¹, especially at about 596 cm⁻¹ and 495 cm⁻¹ corresponding to the stretching collision of O-Mn-O [61] and Mn-O bond stretching vibration which clearly indicate the formation of MnO₂ NPs [66].

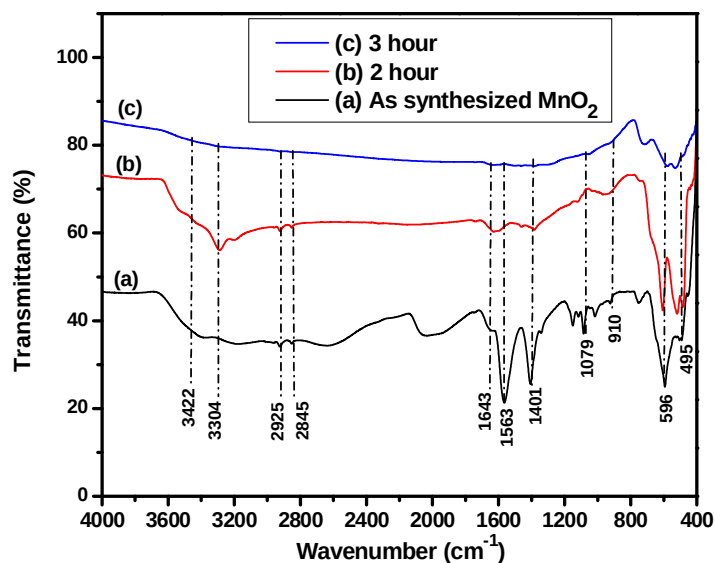


Figure 46. FTIR spectra of (a) as synthesized MnO₂ NPs using *V. amygdalina* leaf, (b) calcined at 450 °C for 2 hour, and (c) calcined at 450 °C for 3 hour.

The FTIR spectrum of α -MnO₂/NiO binary composite is shown in Figure 47. As can be seen from pure α -MnO₂ NPs (Figure 47a), its characteristics peak observed in the band between 600 cm⁻¹ and 475 cm⁻¹ near to 596 cm⁻¹ and 495 cm⁻¹ corresponding to the collision of O-Mn-O and Mn-O bond stretching vibration which indicated the clear formation of α -MnO₂ NPs. The broad absorption peaks which occur at about 3424 cm⁻¹ in the high frequency region attributed to the -OH stretching vibration and the bending mode of physically absorb H₂O molecules (Figure 47b). The peak formed at 1632 and 1089 correspondingly the bending vibration of surface adsorbed water molecules and C-O stretching vibration which might be originating from atmospheric CO₂ adsorption, respectively [69]. The peak at about 532 cm⁻¹ corresponding to the bending vibrations of Ni-O-Ni NPs [216]. The characteristic peaks observed at a wavenumber of 3432 cm⁻¹ and 3303 cm⁻¹ might be assigned as -OH stretching and H-O-H bending vibrations of surface adsorbed water on the hybrid metal oxide NPs when the samples are exposed to the atmosphere (Figure 47c). The weaker but sharp peak at about 1614 cm⁻¹ can be attributed to water molecules bending vibration while the asymmetric band at about 604 cm⁻¹ and 516 cm⁻¹ is associated with Mn-O-Ni stretching vibrations. So, it is clear that an indicated and effective integration between α -MnO₂ and NiO is fabricated to form binary metal oxide NMs.

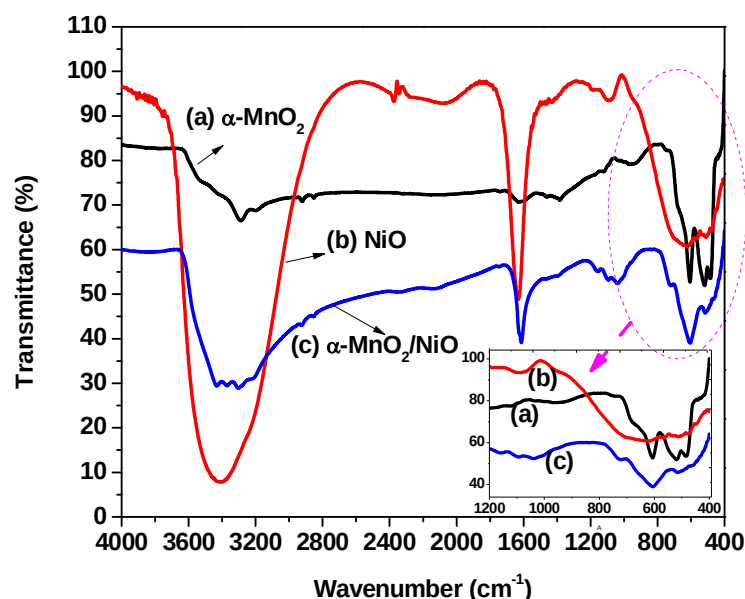


Figure 47. FTIR spectra of α -MnO₂ (a), NiO (b), and α -MnO₂/NiO (c).

The FTIR spectra of pure and α -MnO₂/NiO/PANI ternary nanocomposites are shown and compared in Figure 48. The spectra shown in Figure 48a, demonstrated that a characteristics peak found at 3422, 3304, 596, and 495 cm⁻¹ are attributed to –OH surface water stretching, H-O-H bending, O-Mn-O stretching collision, and Mn-O bond stretching vibrations, respectively. The band found at 3448, 2924, 1636, 1445, 1098, and 969 cm⁻¹ showed N-H stretching, aromatic C-H stretching, quinoid ring stretching vibration, and benzenoid ring vibrations, respectively, as indicated in Figure 48b [217]. The reduction in peak intensity for PANI which is found in α -MnO₂/PANI and the reduction in peak intensity at about 1098 cm⁻¹ and 969 cm⁻¹ as well as new peak appearing at about 508 cm⁻¹ with better in peak intensity than pure PANI indicates the clear transformation of PANI in the nanocomposite as shown in Figure 48c. Moreover, an intense peak found at about 1094 cm⁻¹ and 511 cm⁻¹ illustrates NiO could successfully interfaced in the binary of MnO₂/PANI nanocomposite system as shown in Figure 48d.

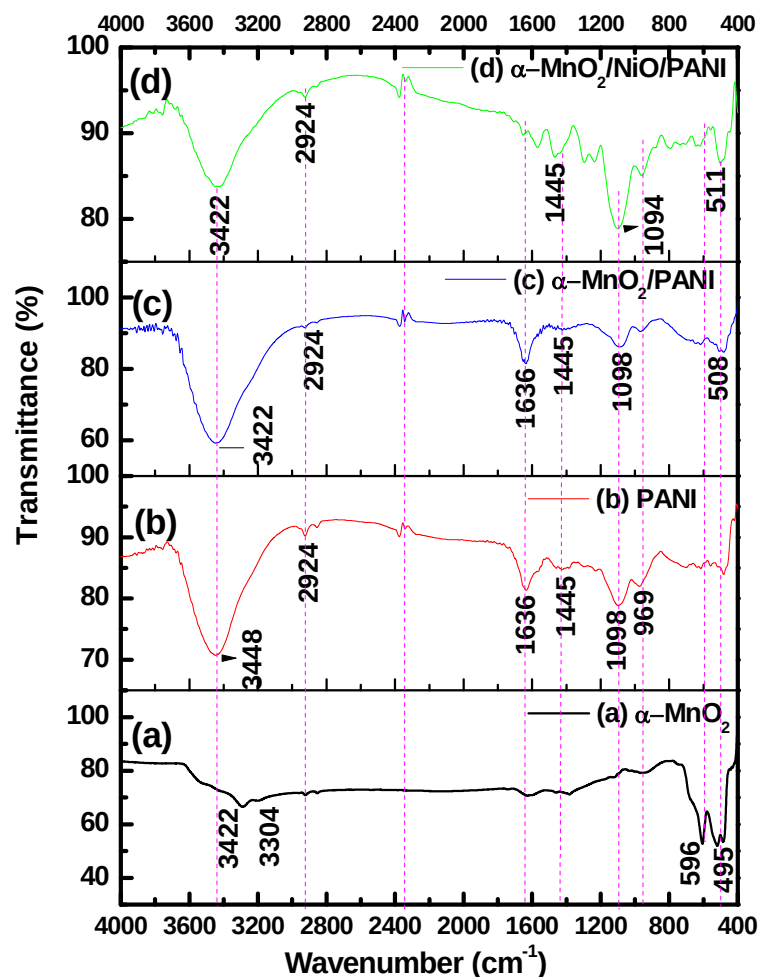


Figure 48. FTIR spectra of α -MnO₂ NPs (a), PANI (b), α -MnO₂/PANI nanocomposite (c), and α -MnO₂/NiO/PANI nanocomposite (d).

4.1.9. Scanning electron microscope (SEM) analysis

The SEM image characterization of α -MnO₂ NPs before and after calcination is shown in Figure 49. The compressed packed microstructure topography presented on Figure 49a was observed from the as synthesized MnO₂ NPs before calcination due to the over growth of organic compounds over it, such existence clearly supported from FTIR and DSC data. Figure 49b and c shows the porous capsule like architecture with fine spherical micrograph having an average crystallite size of ~ 20.28 nm and ~ 20.74 nm for α -MnO₂ NPs synthesized using *V. amygdalina* leaf extract as a reducing and capping agent after 2 and 3 hours calcination time calcined at 450 °C, respectively. Increasing in calcination time increases the degree of surface roughness of the synthesized material (Appendix, Figure A4). So, the

formed NPs is proved from the elemental compositions shown in Figure 49d and confirms the presence of main elements of Mn and O with trace elements K and C.

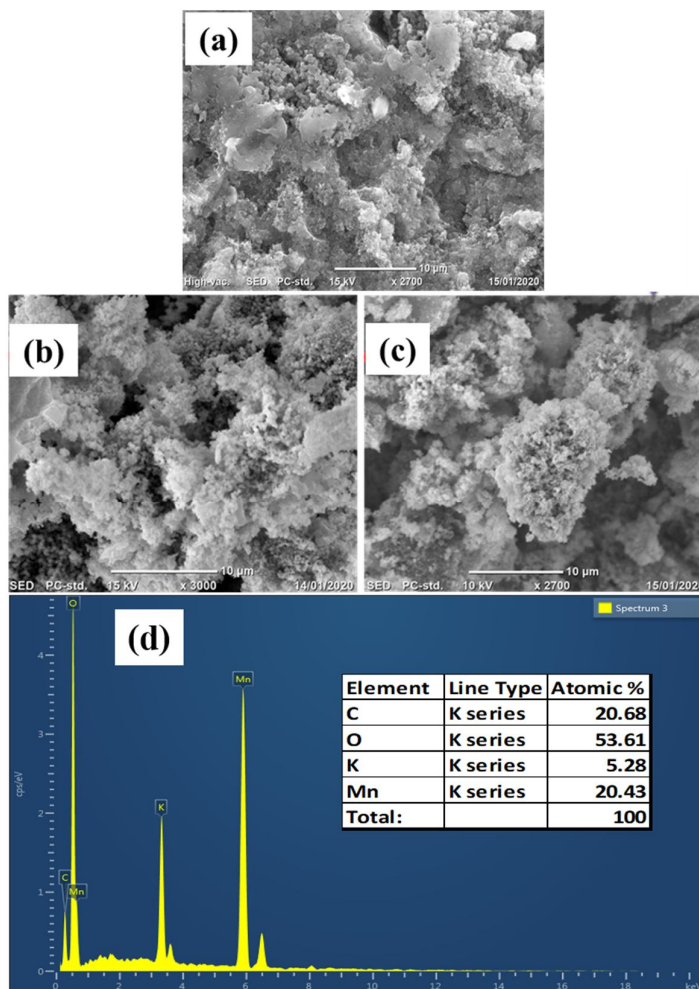


Figure 49. SEM image of as synthesized MnO_2 NPs (a), $\alpha\text{-MnO}_2$ NPs calcined at 450°C for 2 hours (b), $\alpha\text{-MnO}_2$ NPs calcined at 450°C for 3 hours (c), and EDX spectrum (d).

The SEM images of pure and $\alpha\text{-MnO}_2/\text{NiO}$ binary nanocomposites structure were shown in Figure 50. The as prepared pure NiO illustrates that the grain growth are irregularly arranged needle like structure like microstructure as shown in Figure 50a. The formed capsule-layer structures are highly interconnected with each other as shown in Figure 50c. Its morphology contains a fine space, which might be caused by the long time redox reactions between precursor aqueous solutions and *V. amygdalina* leaf extractives. So, the presence of fine cavity or space can be beneficial for the diffusion of electrolytes in the electrochemical reaction system (*i.e.*, it reduces the diffusion length of the electroactive species).

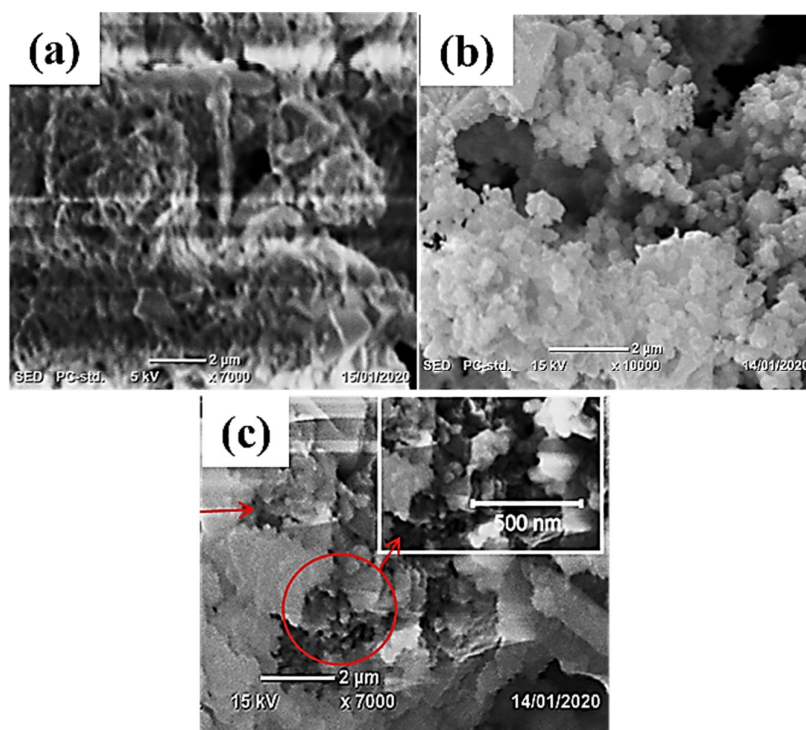


Figure 50. SEM images of NiO (a), α -MnO₂ (b), and α -MnO₂/NiO bimetallic oxide (c) obtained using *V. amygdalina* leaf extract calcined at 450 °C for 2 hours.

The surface topography of α -MnO₂/PANI composite compared to PANI and optimized α -MnO₂ NPs were examined. Figure 51a shows the SEM micrograph for biosynthesized α -MnO₂ NPs using *V. amygdalina* leaf extract. Figure 51b illustrates rough and closely packed granular micrographs for pure PANI with an average crystallite size of 2.18 nm. Careful examination attributed that the type and shape of PANI SEM micrograph is highly depends on oxidant type and aniline monomer concentration. So, when PANI is synthesized using strong oxidant and high aniline concentrations under strongly-acidic conditions (pH < 2.5), globular (granular) type of PANI morphology is synthesized [218]. The clear distribution of α -MnO₂ NPs over PANI granular is shown in Figure 51c. This surface modification with clear α -MnO₂/PANI binary composite formations is proved from the elemental compositions shown in Figure 51d and confirms the presence of main elements, 90.91% (Mn, C, O); dopant elements, 6.39% (S and Cl); and trace, 2.7% (K). Such composites modified with PANI due to its electronic (10^0 - 10^1 S cm⁻¹) and proton conductivities (10^{-9} - 10^1 S cm⁻¹) nature [219], and uniformly distribution of α -MnO₂ in PANI matrix can be considered as a best electrode material in fuel cells.

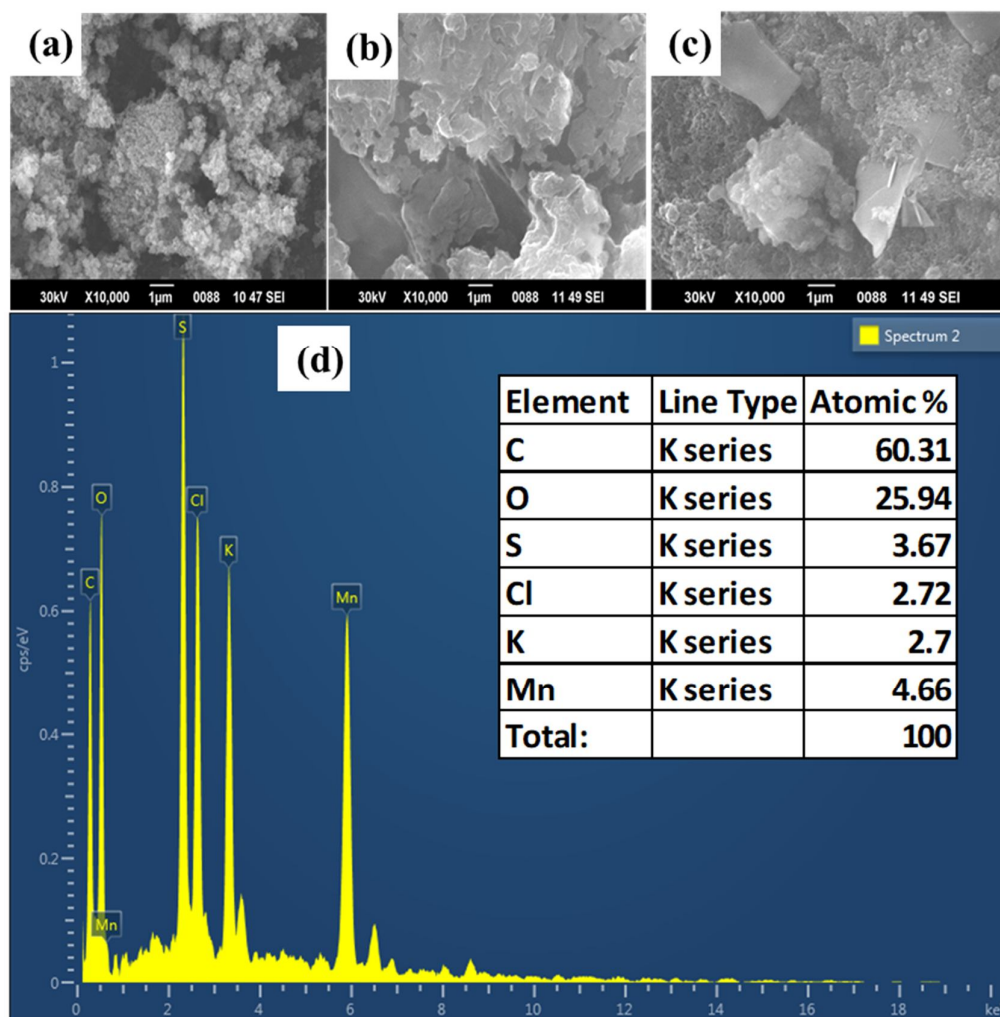


Figure 51. SEM images of α -MnO₂ NPs (a), PANI (b), α -MnO₂/PANI composite (c), and EDX spectrum of α -MnO₂/PANI composite (d).

It is provided that the surface modification and binary composite formations clearly contributes to the better surface size distribution image of ternary α -MnO₂/NiO/PANI (Figure 52a). The incorporation of MnO₂ and NiO NPs could help to change the granular structure of PANI. The elemental composition of this ternary composite is contained from Mn, Ni, C, O and co-dopant elements (S and Cl) with K traces (see Figure 52b). The excess of chlorine resulted from its adsorption on the surface of salt or trapped in this salt as anilinium chloride (emeraldine salt) during the reaction procedure [72]. The presence of sulphur which is originated from APS on the synthesized material improves the electrocatalytic activity on the bare anode [73]. EDX cannot detect trace elements having concentration below 0.01 wt% [107]. So, in this investigation element nitrogen doesn't appear on the spectrum. At low energy resolution, peak overlap may occur between nitrogen

and PANI carbon signal. To prevent this some researchers have used a high energy resolution [108].

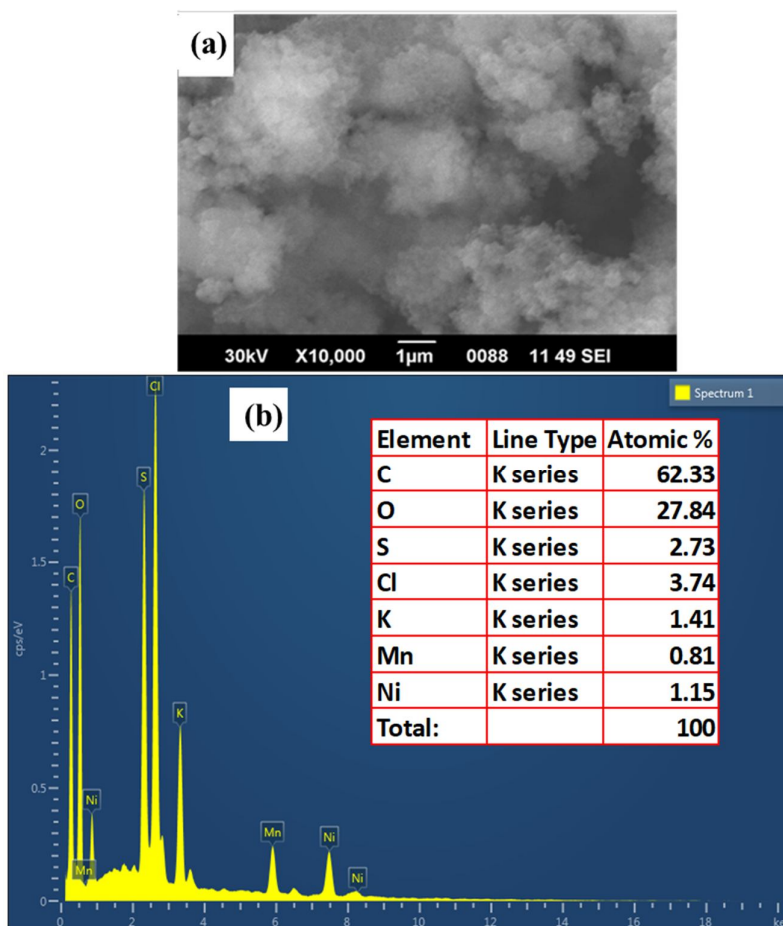


Figure 52. SEM topographies of α -MnO₂/NiO/PANI (a) and EDX spectrum of α -MnO₂/NiO/PANI (b).

4.1.10. Surface roughness and pore size distribution analysis

For this study, the SEM data obtained using a JEOL Ltd. instrument with a model of JCM-6000Plus was loaded to the software (stable version Gwyddion 2.59) and analyzed as shown in Figure 53. According to the International Organization for Standardization (ISO) 4287 standard the pre-selected cutoff frequency (in the units of Nyquist frequency) and profile thickness was specified at 0.02 and 3px (px stands for pixels that determines the margin of an image, 1px = 1/96th of one inch), respectively [220]. So, by using this information, the surface roughness of α -MnO₂, NiO, α -MnO₂/NiO, PANI, α -MnO₂/PANI, and α -MnO₂/NiO/PANI surfaces were represented using 2-D map showed in Figure 53-a1, b1, c1, d1, e1, and f1 while the 3-D map visualize the degree of porous and pore occurrences in all

angles that might show the highest vertical compact surface height followed by visualizing the active surface sites for each catalyst (see Figure 53-a2, b2, c2, d2, e2, and f2 [221]. Values of all surface roughness parameters are given in Table 17. Due to the good surface modification capability of PANI, the R_a value of both α -MnO₂/PANI and α -MnO₂/NiO/PANI composites are 22.92 ± 3.648 nm and 23.65 ± 5.652 nm, respectively and dominated over other catalysts. Even the R_a value of PANI has a higher value than α -MnO₂/NiO nanocatalyst. This shows that the surfaces of PANI aided hybrid composites being rougher than other catalysts.

R_q values of α -MnO₂/PANI and α -MnO₂/NiO/PANI hybrid composites were 28.15 ± 3.645 nm and 28.55 ± 5.617 nm, respectively. Their values were higher than values determined for α -MnO₂, NiO, PANI, and α -MnO₂/NiO nanohybrid, indicating that the degree of surface roughness variation in the PANI based hybrid composites are higher provided that best in α -MnO₂/NiO/PANI composite. The R_{sk} values of α -MnO₂/NiO/PANI shows lower positive value than NiO but has more positive value than the rest catalyst with a value of -0.2178 ± 0.4192 , meaning that composite has more regions of positive curvature than peaks in the surface while values of α -MnO₂ catalyst has least positive (more negative curvature), indicating that there are fewer troughs.

The more the negative values of R_{ku} give information on pore distribution of the sample. Thus from Table 16, the R_{ku} values of α -MnO₂/NiO/PANI catalyst is -0.7148 ± 0.566 while for α -MnO₂/PANI is -0.2429 ± 0.709 and for PANI is -0.5079 ± 0.342 . The results show that the pore size distribution by MnO₂/NiO/PANI nanocomposite is more scattered (*i.e.*, it is not compact or more concentrated) while the pore size distribution of α -MnO₂ catalyst is more concentrated due to the more positive value of 0.9627 ± 1.798 . This is showing that the surfaces roughness of α -MnO₂/NiO/PANI is better in surface area and reactive surface site than PANI and α -MnO₂/PANI due to the successfully integration between α -MnO₂, NiO NMs, and PANI matrix [222]. R_{ku} value is exactly proved from R_q values, which indicates the degree of surface roughness variation found on α -MnO₂/PANI and α -MnO₂/NiO/PANI have higher and best in roughness property than other catalysts as clearly shown from Table 16. In addition, the dispersion of metallic oxide nanocatalysts with PANI matrix could also enhances the mechanical properties of the synthesized nanocomposites [223] and their thermal stability which was supported from TGA of α -MnO₂ NPs. Not only these, the addition of α -MnO₂ NPs and α -MnO₂/NiO bimetallic oxides on polymer matrix increased surface roughness, increase multi-functionality by changing shapes of the materials [224].

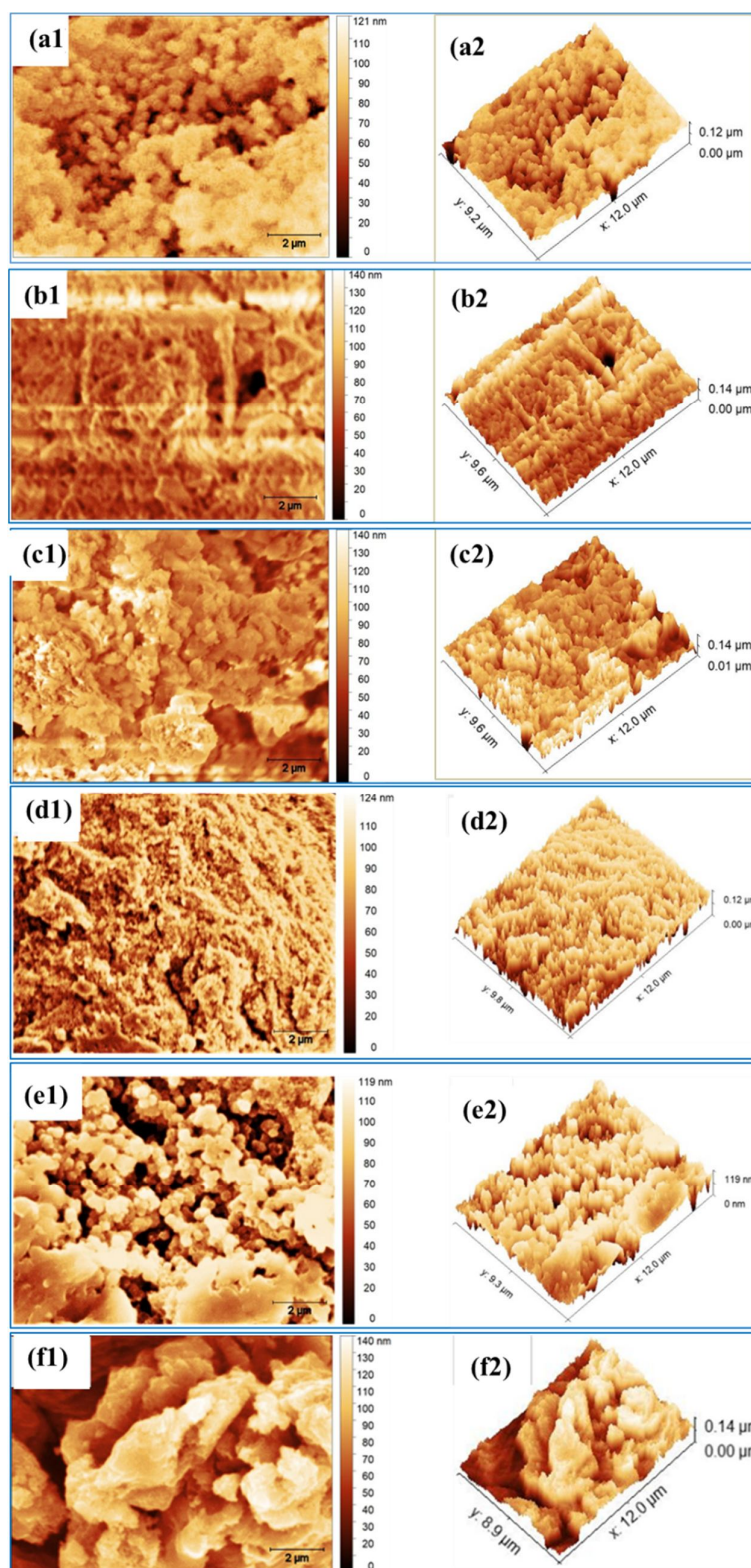


Figure 53. 2-D and 3-D SEM images of (a1) and (a2) from α -MnO₂, (b1) and (b2) from NiO NPs, (c1) and (c2) from α -MnO₂/NiO bimetallic oxides, (d1) and (d2) from PANI, (e1) and (e2) from α -MnO₂/PANI, and (f1) and (f2) from α -MnO₂/NiO/PANI catalysts.

Table 16. Surface roughness parameter results for α -MnO₂, NiO, PANI, α -MnO₂/NiO, α -MnO₂/PANI, and α -MnO₂/NiO/PANI catalysts.

Catalyst	R_a (nm)	R_q (nm)	R_{sk}	R_{ku}
α -MnO ₂	13.11 $\pm$ 3.163	17.21 $\pm$ 3.587	-0.8903 $\pm$ 0.5439	0.9627 $\pm$ 1.798
NiO	12.90 $\pm$ 2.576	16.58 $\pm$ 3.414	-0.1055 $\pm$ 0.4877	0.1588 $\pm$ 0.6954
α -MnO ₂ /NiO	14.91 $\pm$ 2.948	19.05 $\pm$ 3.182	-0.2267 $\pm$ 0.4854	0.2018 $\pm$ 0.7333
PANI	19.96 $\pm$ 2.264	24.22 $\pm$ 2.423	-0.3789 $\pm$ 0.1872	-0.5079 $\pm$ 0.342
α -MnO ₂ /PANI	22.92 $\pm$ 3.648	28.15 $\pm$ 3.645	-0.6072 $\pm$ 0.3500	-0.2429 $\pm$ 0.709
α -MnO ₂ /NiO/PANI	23.65 $\pm$ 5.652	28.55 $\pm$ 5.617	-0.2178 $\pm$ 0.4192	-0.7148 $\pm$ 0.566

It is noted that watershed method is more realistic techniques for pore and their distribution determination in the range between 1 nm and 200 nm while the rest method have a large range of size with more complicated structural determination including pore with bigger in size [109]. So that based on the principle of water flows to a region with a local minimum which represents a plane pore. The method has two specific processes, such as water drops to each point on the surface of the catalyst and water flows to the region of the local minimum. Then the pores are identified with the pore size, pore surface area, and pore volumes were determined by the volume of water converging to the local minimum region.

Based on the supported R_{ku} values, pores associated with α -MnO₂, PANI, α -MnO₂/PANI, and α -MnO₂/NiO/PANI catalysts are shown in Figure 54-a1, b1, c1, and d1 and their detailed pore parameters are shown in Table 17. The pore size distribution are shown in Figure-a2, b2, c2, and d2. Average mean pore size formed by α -MnO₂/PANI nanocomposite has a minimum value of 45.81 $\pm$ 12.16 nm while the highest average mean pore size of 57.84 $\pm$ 21.92 nm was for α -MnO₂/NiO/PANI composite. By comparison, α -MnO₂/NiO/PANI catalyst (29.91 μm^{-1}) has more surface area to volume ratio than MnO₂, PANI, and α -MnO₂/PANI catalysts with a value of 17.88, 23.09, and 24.08 μm^{-1} , respectively that clearly showed in Table 17. It is concluded that the synthesized materials covered a size from near macro to highest meso pore size distribution range as compared with other related work [225–228].

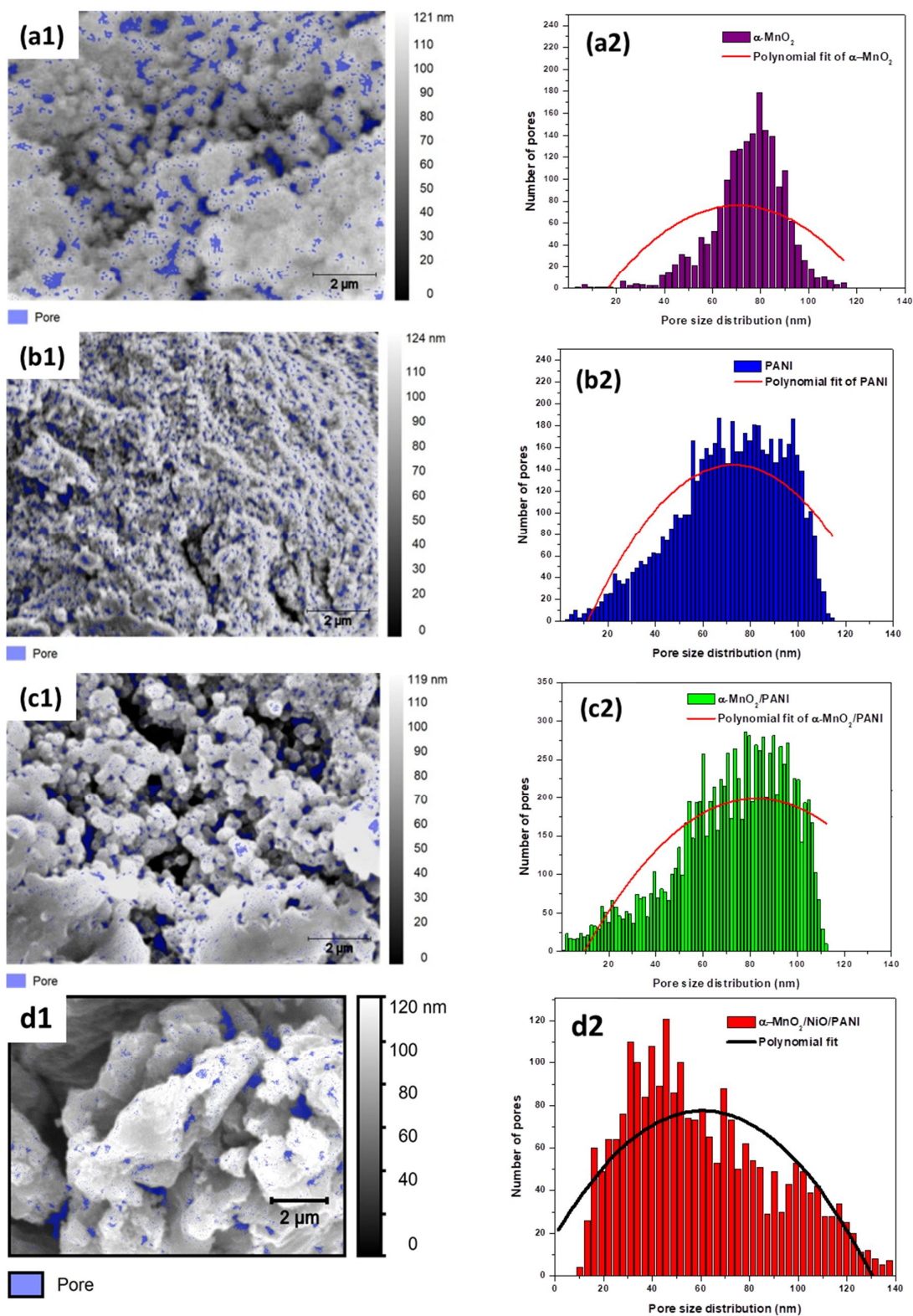


Figure 54. SEM images of (a1) α -MnO₂, (b1) PANI, (c1) α -MnO₂/PANI, and (d1) α -MnO₂/NiO/PANI. The histogram of average pore size distributions of (a2) α -MnO₂, (b2) PANI, (c2) α -MnO₂/PANI, and (d2) α -MnO₂/NiO/PANI catalysts results by the Watershed.

Table 17. Pore structure statistics quantities for different catalysts by the Watershed method.

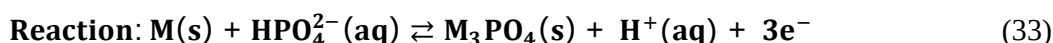
Catalyst	Number of pores (Sum)	Average Mean pore size (nm)	Surface area to volume ratio (μm^{-1})
α -MnO ₂	1841	57.13 ± 13.62	17.88
PANI	5838	46.78 ± 6.869	23.09
α -MnO ₂ /PANI	10153	45.81 ± 12.16	24.08
α -MnO ₂ /NiO/PANI	2374	57.84 ± 21.92	29.91

4.1.11. Electrochemical characterizations

Electrochemical characterizations were performed using three electrode electrochemical workstation (BioLogic SP-300, Canada) integrated with EC-Lab software. The electrocatalytic activities of bare PGE and those modified with α -MnO₂, NiO, α -MnO₂/NiO, PANI, α -MnO₂/PANI, and α -MnO₂/NiO/PANI nanocatalyst were evaluated using cyclic voltammetry under phosphate buffer electrolyte solution in the potential range between -0.8 V and +0.8 V at the scan rate of 50 mV s⁻¹. The clear and defined redox peaks were observed for all electrodes shown in Figure 55a. Among, peak current observed using bare PGE was lower than the modified electrodes, due to its limited oxidation and reduction reaction in its surface resulting from the slow mass transfer rate. Such limited oxidation and reduction reaction store lower charge storage capacity as shown in Figure 55b. So, its electrocatalytic performance was improved with modification by α -MnO₂, NiO, α -MnO₂/NiO, PANI, α -MnO₂/PANI, and α -MnO₂/NiO/PANI as observed from the increased peak currents shown in Figure 55a. An increase in both anodic and cathodic peak currents from mono catalytic to composite modified PGE indicates excellence in oxidation and reduction reaction on the electrode, respectively, and the best electrode that showed better mass transfer efficiency and reversibility was found on α -MnO₂/NiO/PANI followed by α -MnO₂//PANI. If the peak current is high, the diffusion rate is large. Therefore, the best-modified electrode is the one that has a better peak current values [229]. This activity increases the charge transfer performance of α -MnO₂/NiO and α -MnO₂/PANI nanocatalyst modified PGE as shown in Figure 55b. Provided that the clear integration of biosynthesized α -MnO₂/NiO in conducting PANI matrix exhibited maximum peak currents with highest in charge transferring performance on α -MnO₂/NiO/PANI modified PGE and this improves its electrical conductivity and conduction channels.

The peak to peak potential separation (ΔE_p) was lower in PANI encapsulated α -MnO₂/NiO composite and α -MnO₂ modified PGE than other. From this order α -MnO₂/PANI/PGE has a minimum value of 0.216 V while bare PGE has a biggest value of 0.257 V. The minimum

ΔE_p enhance electrocatalytic activity on the modified PGE. It indicated that α -MnO₂/NiO/PANI/PGE exhibited a fast electron transfer rate that enables to increase electrical conductivity due to its lower ΔE_p . So, the minimum ΔE_p indicated the fast electron transfer movement between electroactive species and exposed surface area of electrode interfaces. This improvement exactly supported from the high surface roughness degree and better pore structure distribution coverage. The shift in anodic peaks potential from PGE to α -MnO₂/NiO/PANI/PGE to towards more negative potential showed that the result of higher conductivity. So, the degree of oxidizing ability was increases from bare PGE to α -MnO₂/NiO/PANI/PGE (see Table 18). Catalyst in this reactions may undergo hydrogen ion desorption accompanied by formation of poorly soluble phosphate films as proved by the literature [230]. So, the possible oxidation reaction and their reversible reduction responses are given by the equation [230].



where M stands for all catalysts.

Generally, from Table 18, the peak to peak voltage potential of all electrodes are higher than 59/n mV ($\Delta E_p > 59/n$ mV). This is indicating that the peak currents are controlled by both charge transfer and mass transport processes, which is one of the primary indications of a quasi-reversible system which was supported by other work [231].

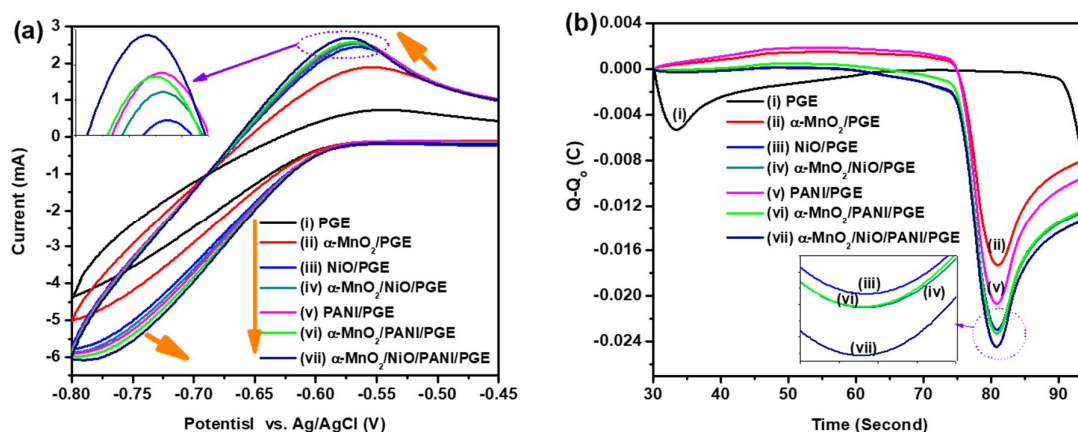


Figure 55. (a) Cyclic voltammogram and (b) total charge exchange of [(i) PGE, (ii) (α -MnO₂/PGE, (iii)) NiO/PGE, (iv) α -MnO₂/NiO/PGE, (v) PANI/PGE, (vi) α -MnO₂/PANI/PGE, and (vii) α -MnO₂/NiO/PANI/PGE] in 100 mM PBS (pH = 7.4) at a scan rate of 50 mV s⁻¹.

Table 18. Summary of redox current, peak potential, and charge storing capability on different catalyst modified PGE.

Electrode	I_{pa} (mA)	I_{pc} (mA)	E_{pa} (V)	E_{pc} (V)	ΔE_p (V)	(Q-Q _o) (mC)
PGE	0.735	-4.379	-0.543	-0.8	0.257	5.291
α -MnO ₂ /PGE	1.897	-5.015	-0.553	-0.799	0.246	17.267
NiO/PGE	2.442	-5.785	-0.565	-0.800	0.235	22.941
α -MnO ₂ /NiO/PGE	2.522	-5.875	-0.566	-0.799	0.233	23.28
PANI/PGE	2.579	-5.895	-0.568	-0.798	0.230	20.659
α -MnO ₂ /PANI/PGE	2.569	-5.975	-0.57	-0.794	0.224	23.258
α -MnO ₂ /NiO/PANI/PGE	2.685	-6.079	-0.573	-0.789	0.216	24.456

A pair of quasi-reversible redox peak was dominated on the binary α -MnO₂/PANI and ternary α -MnO₂/NiO/PANI nanocomposite modified PGE from the best fit-line of I_p versus $v^{1/2}$. The reversibility of these peaks suggests that the fabricated nanocomposite do not easily decompose during charge transfer mechanism and this helps as a medium for stable and fast electron transfer ability. A pair of weak redox peak was observed at bare PGE with anodic (I_{pa}) and cathodic peak current (I_{pc}) values of 0.735 mA and 4.379 mA, respectively, which was the least among other modified electrodes as shown in Figure 56a. Since the effective surface areas (effective electroactive area) of the electrode is a main factor which affects its electrocatalytic activity performance in the electrochemical based system. So, to prove this area, many experimental works which is governed by quasi-reversible reaction at each electrode have been followed *Randles-Sevcik* equation from the direct relation between anodic peak current with the square root of scan rate [232–234].

$$I_p = (2.69 \times 10^5)n^{3/2}D^{1/2}v^{1/2}AC \quad (34)$$

where I_{pa} is anodic peak current (A), n is number of electron transfer, D is the diffusion coefficient of HPO_4^{2-} ($7.60 \times 10^{-6} \text{ cm}^2/\text{s}$ [235]), v is the scanning rate (V s^{-1}), A is effective area of electrode (m^2), and C concentration of PBS (mol cm^{-3}). Their effective areas were described to be 0.00269, 0.00695, 0.00945, 0.00942, and 0.00984 cm^2 for bare PGE, α -MnO₂/PGE, PANI/PGE, α -MnO₂/PANI/PGE, and α -MnO₂/NiO/PANI/PGE, respectively at 0.05 V s^{-1} . These results, suggests that α -MnO₂/NiO/PANI nanocomposite

modified PGE has the largest effective surface area which helps to facilitate a fast electrocatalytic activity as observed from its high peak current. These increments may be attributed to the large effective surface area of PANI which anchored more adsorption and desorption of phosphate molecules as well as high polarons mobility due to the presence of NiO. It is expected that the α -MnO₂ and α -MnO₂/NiO dispersed (embedded) in PANI matrix interacts with lone pairs of nitrogen atoms of PANI, thus increasing the number of polarons in PANI and creating a more number of active sites at a large surface area for the interaction of lone pairs of phosphate molecules to polarons of PANI. Evidences in the composite system suggests that the role of metal oxide NPs in the conducting polymer based binary and ternary system could act as a bridge during charge transport between the polymer and carbon electrode [210].

Hence in this study the role of α -MnO₂ and NiO NPs acts as a bridge between PANI and PGE, both of them helps in the movement of charge carriers from PANI to PGE and thus increases electrical conductivity because of a greater mobility of charge carriers along the π -conjugated system of PGE. Thus, α -MnO₂ and α -MnO₂/NiO NPs bridges additionally facilitate the hopping movement of charge carriers between PANI and PGE in the α -MnO₂/PANI/PGE and α -MnO₂/NiO/PANI/PGE nanocomposites, respectively. To further understand the redox reaction effect at different scan rate on α -MnO₂/PANI and α -MnO₂/NiO/PANI nanocomposites modified PGE are discussed in brief on the following sections.

The effect of scan rate on the produced peak current by α -MnO₂/PANI hybrid composite modified PGE is shown in Figure 56a. It was performed at scan rates of 5, 10, 20, 50, 100, and 200 mV s⁻¹. The obtained voltammograms show that the produced peak current was enhanced with increasing scan rate. As scan rate increases, sharper in cathodic peak current response accompanied with intense sensitivity was obtained at higher scan rates, which is assumed to be a strong interaction results the faster rate of electron transfer between soluble phosphate films and α -MnO₂/PANI/PGE. The dependence of plot of anodic and cathodic peak currents as a function the scan rates shown in Figure 56b provides a nonlinear relationship with R² equal to 0.84117. This result shows that both oxidation and reduction reactions on α -MnO₂/PANI/PGE surface is not fully under surface adsorption controlled process. On the other hand, the plots of the anodic and cathodic peak currents were linearly dependent on the square root of the scan rate shown in Figure 56c. This indicates that the redox process occurring on the electrode surface is near-reversible and diffusion controlled

process [236]. The result demonstrated that a linear relationship with the oxidation of buffer solutions by producing H^+ ion to the solution on $\alpha\text{-MnO}_2/\text{PANI}/\text{PGE}$ has controlled by diffusion process [237]. It is concluded that, if the redox reaction is diffusion controlled, the peak current should be linearly proportional to square root of scan rate [238], and negative intercept = -0.37604 with $R^2 = 0.97419$ from logarithm of the scan rates *versus* logarithms of anodic peak current are shown in Figure 56d which suggests there might be some adsorption intermediate in the process [239]. As discussed in Figure 56b and d, the electrochemistry of the hydrogen ion oxidation at $\alpha\text{-MnO}_2/\text{PANI}/\text{PGE}$ might be also controlled by adsorption process. Hence, there is a minimal possibility to diffuse the phosphate at $\alpha\text{-MnO}_2/\text{PANI}/\text{PGE}$ surface. As a result, the reversibility of hydrogen ion desorption was affected at higher scan rates.

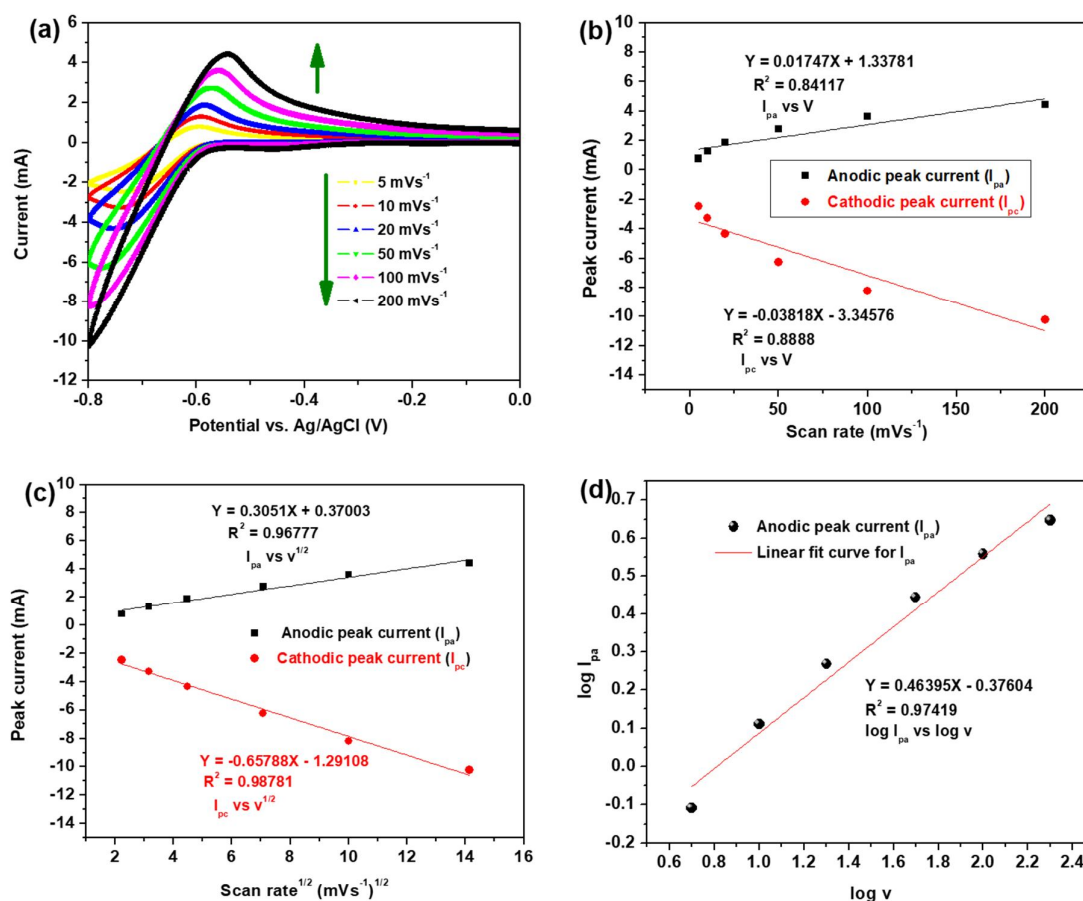


Figure 56. (a) Cyclic voltammograms of $\alpha\text{-MnO}_2/\text{PANI}/\text{PGE}$ electrode recorded at scan rates of 5-200 mV s^{-1} (inner to outer), (b) plot of peak currents *versus* scan rates, (c) variation of peak currents *versus* square root of scan rate, and (d) variation of $\log V$ *versus* $\log I_{pa}$.

The possible redox reactions found at α -MnO₂/NiO/PANI modified PGE is also followed reversible process [230]. As observed from its cyclic voltammograms, α -MnO₂/NiO/PANI nanocomposite modified PGE was the best electrode among the rest. Therefore, to see the effect of scan rate on the peak current and peak potential of α -MnO₂/NiO/PANI/PGE is shown in Figure 57a at the scan rates (5-200 mVs⁻¹). The responses of both I_{pa} and I_{pc} currents was gradually increased with increasing scan rate from 5 mV s⁻¹ to 200 mV s⁻¹. Like α -MnO₂/PANI/PGE, as scan rate increases, sharper in cathodic peak current response accompanied with intense sensitivity was obtained at higher scan rates, which is assumed to be a strong interaction results for the faster rate of electron transfer between poorly soluble phosphate films and α -MnO₂/NiO/PANI/PGE. Figure 57b justified that the linear dependence of both I_{pa} and I_{pc} *versus* square root of scan rate with linear regression equation of I_{pa} = 0.30405v^{1/2} + 0.29912, R² = 0.97 and I_{pc} = -0.63452 v^{1/2} - 1.1702, R² = 0.98. The dependence of peak potentials (E_{pa} and E_{pc}) on scan rate is also shown in Figure 57c. Hence implying that the nature of redox process occurring on the electrode surface is nearly reversible (*i.e.*, quasi-reversible) [236] and clearly diffusion controlled process [237]. The anodic peak potential (E_{pa}) was highly correlated with natural logarithm of scan rate (ln v) shown in Figure 57d. To evaluate the electrochemical parameters from such linearity, Laviron equation was applied as follows [240]:

$$E_{pa} = E^0 + \frac{RT}{(1-\alpha)nF} + \frac{RT}{(1-\alpha)nF} \ln v \quad (35)$$

where E⁰ is the formal potential ($\frac{E_{pa} + E_{pc}}{2}$), α is the charge transfer coefficient of the system, v is the scan rate, n is the number of electron transfer R is universal gas constant (8.314 Jmol⁻¹ K⁻¹, T = 298 K, F = 96,493 C mol⁻¹, and k_s is the heterogeneous electron transfer rate constant. From the slope of E_{pa} *versus* lnv, $\alpha = 0.51$. So, if α is closer to 0.5 the electrode reaction is quasi-reversible and governed by diffusion controlled process [241]. Laviron equation [242], was applied to calculate k_s for each electrode as well as electron transfer at each scan rate.

$$\log k_s = \alpha \log(1 - \alpha) + (1 - \alpha) \log \alpha - \log \frac{RT}{nFv} - \frac{\alpha(1-\alpha)nF}{2.3RT} \Delta E_p \quad (36)$$

The calculated k_s value of α -MnO₂/NiO/PANI/PGE at constant scan rats of 50 mV s⁻¹ was higher than the rest of the electrode and this indicated that α -MnO₂/NiO/PANI nanocomposite is a good facilitator for direct electron transfer between electroactive species and bare PGE (see Table 19). The direct dependence of k_s over peak potential is also given

in Table 20 and from this, ΔE_p of the electrode increases with increasing scan rates, indicating that the charge transfer at the α -MnO₂/NiO/PANI modified PGE is quasi-reversible as evidenced from other work [243].

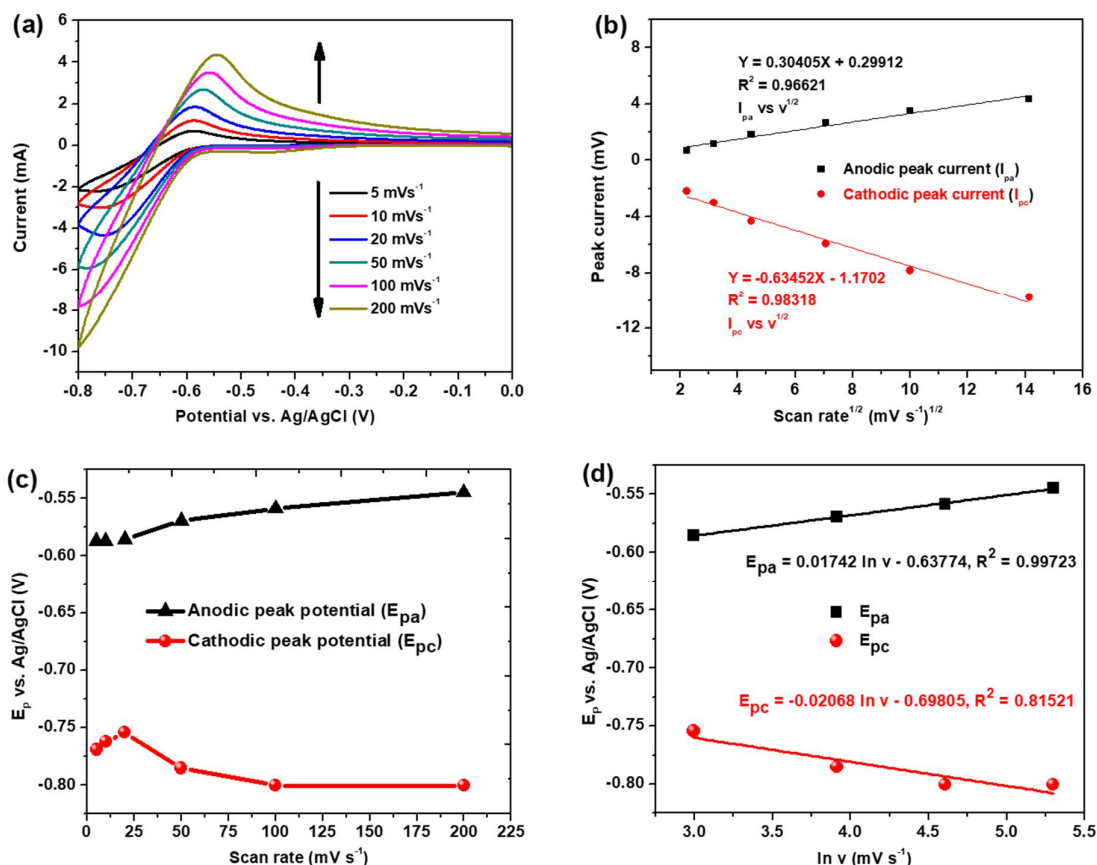


Figure 57. (a) Cyclic voltammograms of α -MnO₂/NiO/PANI/PGE in 100 mM PBS (pH = 7.4) at different scan rates, (b) linear plots of peak currents *versus* the square root of scan rate ($v^{1/2}$), (c) linear plots of peak potential *versus* scan rates, and (d) linear plots of peak potential *versus* natural logarithm of scan rate ($\ln v$).

Table 19. Summary of heterogeneous electron transfer rate constant and peak separation for PGE, α -MnO₂/PGE, PANI/PGE, α -MnO₂/PANI/PGE, and α -MnO₂/NiO/PANI/PGE anode at a scan rate of 50 mVs⁻¹.

Electrode	$\Delta E_p = E_{pa} - E_{pc}$ (V) <i>versus</i> Ag/AgCl	k_s (s ⁻¹)
PGE	0.257	2.91776971
α -MnO ₂ /PGE	0.246	2.917832732
PANI/PGE	0.23	2.917924402
α -MnO ₂ /PANI/PGE	0.224	2.91795878
α -MnO ₂ /NiO/PANI/PGE	0.216	2.918004616

Table 20. A heterogeneous electron transfer rate constant and peak separation for α -MnO₂/NiO/PANI/PGE at different scan rate.

Scan rate (m Vs ⁻¹)	5	10	20	50	100	200
$\Delta E_p = E_{pa} - E_{pc}$ (V) versus Ag/AgCl	0.182	0.175	0.169	0.216	0.242	0.256
k_s (s ⁻¹)	0.292	0.584	1.167	2.918	5.836	11.671

EIS measurements were caused to analyze surfaces of electrodes and their interfacial properties from Nyquist, admittance, bode-magnitude, and bode-phase plots in 100 mM PBS (pH = 7.4) electrolyte solution (Figure 58). The charge carrier transfer between electrode and electrolyte interfaces was understood in the frequency range of 10 mHz -200 kHz at 0.252 V vs Ag/AgCl. The diagrams from Nyquist plot estimates quantities of important elements, such as ionic charge-transfer resistance (R_s), interfacial charge-transfer resistance (R_{ct}), double layer capacitance (C_{dl}), and Warburg impedance/activation or concentration loss (Z_w) of the equivalent circuit by using EC-Lab Zsim analysis. For this study, a representation of Randles type circuit model shown in the Nyquist plots was fitted for all EIS experimental data. The model defined that semicircle region found at high frequencies in the diagram provides information about the electron transfer limited process while linear region from low frequencies may associated with Warburg diffusion process [237]. The length of the diameter from the semicircle showed surface/or interface charge transfer resistance that defines the dielectric and insulating properties of the electrode/electrolyte solution interface [244].

The Nyquist plots of bare PGE and modified PGE are shown in Figure 58a. The determined EIS parameters modelled from the simulation equivalent circuit analysis are given in Table 22. The values of R_{ct} for α -MnO₂/PANI/PGE and α -MnO₂/NiO/PANI/PGE are relatively smaller than the others due to the high conductivity of PANI obtained in PBS. Off course, least in R_{ct} is found in α -MnO₂/NiO/PGE is due to the successful integration between α -MnO₂ and NiO as well the fast charge/discharge mechanism nature of NiO NMs as supported by other related work [245], provided that its fast electron transfer rate efficiency is no longer due to having higher R_s value and smaller charge storing capacity than α -MnO₂/PANI/PGE and α -MnO₂/NiO/PANI/PGE as evidenced from CV test. Hence, the smaller R_s found on α -MnO₂/PANI/PGE and α -MnO₂/NiO/PANI/PGE with a values of 8.86 Ω and 9.26 Ω , respectively, are lower than the rest of the electrodes due to their synergistic effect between α -MnO₂, NiO, and PANI. This lower value in both interfacial and ionic charge-transfer resistance promoting a rapid electron transfer in the PBS by lowering the intrinsic resistance at higher frequency range shown in Figure 58b. Which are consistent

with the CV tests. This provides a fast diffusion of the electrolyte from its bulk into the surfaces of electroactive materials leads R_s become small which was supported from Z_w results shown in Table 21. The double layer capacitance or constant phase elements of α - MnO_2/PANI modified PGE is the highest due to having the least degree of polarization and Ohmic resistances (low magnitude of impedance) properties as shown in Figure 58b.

From $\log |Z|$ as a function of $\log f$ shown in Figure 58b elucidated that α - MnO_2/PANI modified PGE exhibited lowest $\log |Z|$ value of 0.94 Ω at 19.15 kHz, indicating that fast electron transfer rate is accomplished compared to other electrodes (see Table 21). The typical capacitive and resistive behaviors of the prepared electrodes can also be evidenced from the frequency dependent phase angle (ϕ) plot presented in Figure 58c. ϕ of 90° - 45° shows capacitive behavior while 45° - 0° range attributed to the resistive behavior, and -90° conforms an ideal supercapacitor [246]. So, at the low frequency, the lines of α - $\text{MnO}_2/\text{PANI}/\text{PGE}$ and α - $\text{MnO}_2/\text{NiO}/\text{PANI}/\text{PGE}$ exhibit a phase angle with a values of -73.182° and -73.048° , respectively, than others. Thus their optimum ϕ are found in the range between -45° and -90° . This result confirms the electrochemical property improvement with better charge storing capacitance behavior, which are consistent with the CV results.

The admittance plots of bare PGE and catalyst modified PGE were done to easily understand the conductivity of materials. The highest conductivity was obtained for α - $\text{MnO}_2/\text{PANI}/\text{PGE}$ that exhibited with a magnitude of conductance ($|Y|$) value 126 mS as shown in Figure 58d and Table 21. All the properties studied by impedance have a clear relation with results obtained from CV analysis.

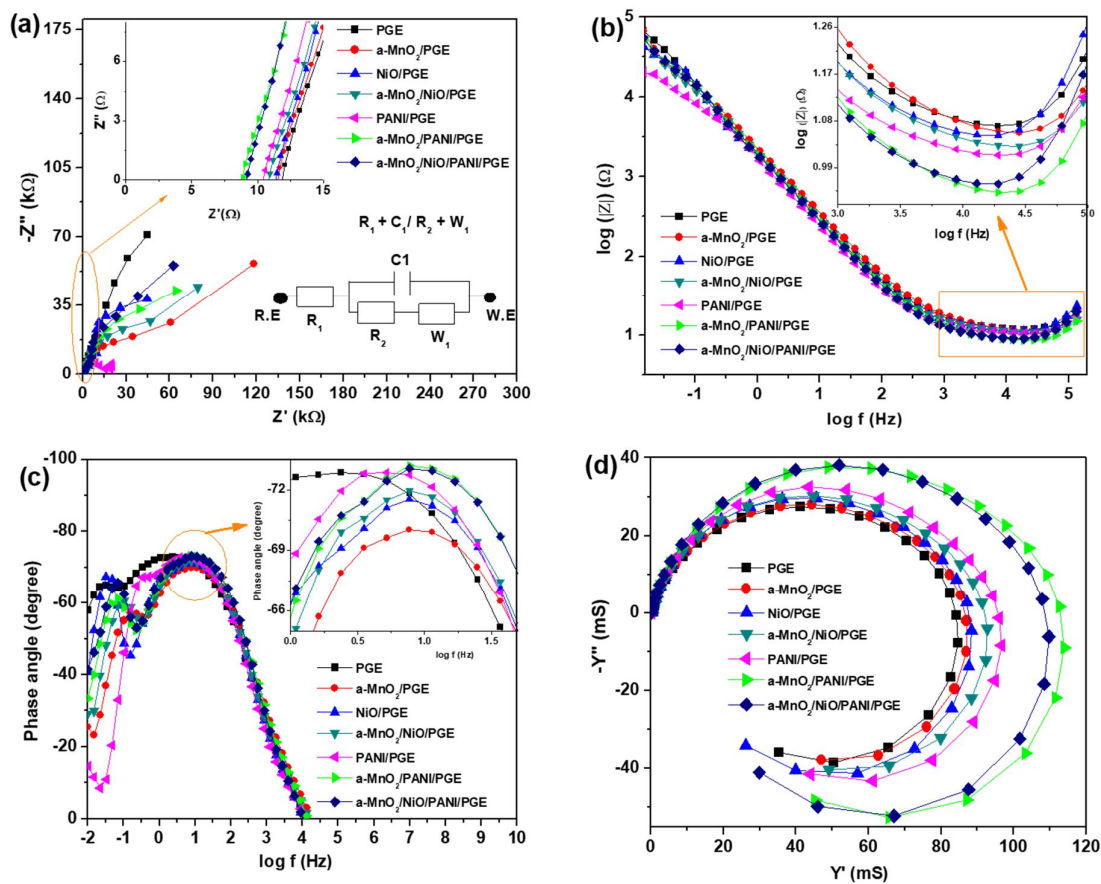


Figure 58. (a) Nyquist and the inset displayed the equivalent circuit (Randles circuit model with a reference electrode (R.E) and working electrode (W.E)), (b) Bode-magnitude, (c) Bode-phase, and (d) Admittance plots of different electrodes.

Table 21. EIS parameters of bare PGE and different catalyst modified PGE.

Electrode	R_1 (Ω)	R_2 (Ω)	C_1 (F)	W_1 ($\Omega \text{ s}^{-1/2}$)	$ Y $ (mS)	$\log Z $ (Ω)	θ (degree)
PGE	11.91	1519	5.12×10^{-5}	25283	92	1.07	-72.851
α -MnO ₂ /PGE	11.54	1119	7.42×10^{-5}	13283	95	1.06	-70.039
NiO/PGE	11.24	1531	6.37×10^{-5}	7250	94	1.05	-71.528
α -MnO ₂ /NiO/PGE	10.84	300.6	3.00×10^{-4}	7109	102	1.03	-71.935
PANI/PGE	10.47	1071	8.50×10^{-5}	736.1	106	1.01	-72.854
α -MnO ₂ /PANI/PGE	8.86	958	8.01×10^{-4}	448.5	126	0.94	-73.182
α -MnO ₂ /NiO/PANI/PGE	9.26	788.9	9.26×10^{-5}	12000	121	0.96	-73.048

$R_1 = R_s$, $R_2 = R_{ct}$, $C_1 = C_{dl}$, $W_1 = Z$

4.2. Electrical energy generation and biodegradation test

4.2.1. Process parameter optimization

Before justify the catalyst modified electrode in MFC setup, the favorable conditions of *E. coli* was optimized for effective performances. Thus, experiments were divided into two main series in order to evaluate the interaction of operational conditions on doubled chamber MFC, which predominantly affect bioremediation followed by electricity generation performances, including pH and operating temperature were examined. It is presumed that pH of aqueous environment has a significant influence on the rate of wastewater treatment and power density in MFCs, by affecting the physiology of bacteria [247]. In a dual-chamber MFC with synthetic wastewater, investigations showed that the suitable pH for maximizing performance was near neutral [248]. However in another study, an acidophilic operational condition at pH around 6 was obtained as the optimum condition for effective bioelectricity generation and wastewater treatment efficiency [249]. So, to find the optimum pH, which was expected to help the required activity of *E. coli* for better current intensity with efficient power density production that are responsible for effective bioremediations are performed and data are presented in Figure 59. As the graph demonstrates, power and current densities were reasonably low at pH 4.45 and followed by a drastically increased pH range between 5.52 and 7.42. The power density was increased from 1.305 mW m⁻² to 13.463 mW m⁻² at pH 5.52 and 7.42, respectively. However, it was not further increased at pH higher than 7.42. Therefore, power density dropped to 1.611 mW m⁻² at pH 8.67. A similar trend was also followed for current density. Other than 7.42 the rest pH influences nutrient absorption and its solubility by *E. coli* cell membrane and this directly impressed microbial community structure involved in biodegradation as evidenced from the reference [247]. As a result, pH close to 7.42 is considered as the promising environmental condition for *E. coli* growth on PGE.

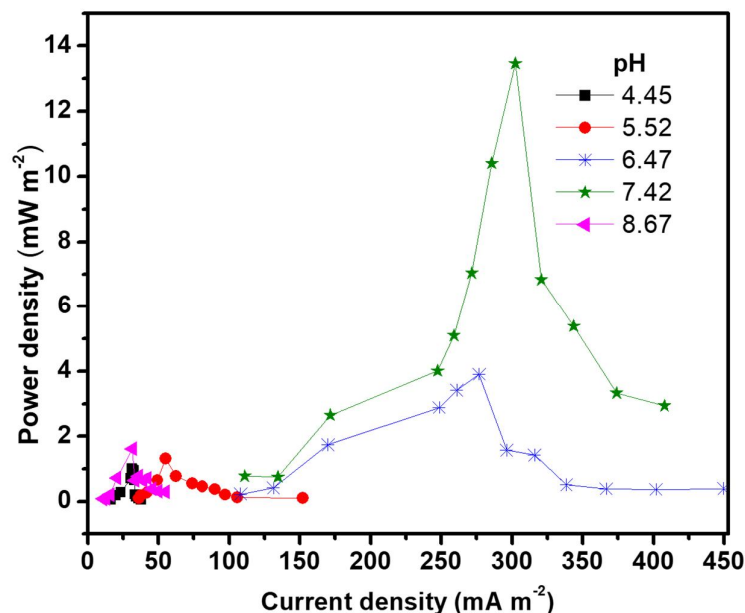


Figure 59. Power density as a function of current density using *E. coli*, $R = 500 \, \Omega$, $T = 26 \, ^\circ\text{C}$, glucose = $100 \, \text{mg L}^{-1}$.

Optimizing temperature is also another crucial factor for maximum coulombic and energy production efficiencies during biodegradation. Depending on the types of substrate (either single or complex) and microorganism like psychrophilic ($23 \pm 3 \, ^\circ\text{C}$) and mesophilic ($35 - 40 \, ^\circ\text{C}$), and thermophilic (up to $50 \, ^\circ\text{C}$) has been found to be the optimum condition for most of the anaerobic digesters [250]. So, in this study stepwise increasing of operating temperature from 25 to $40 \, ^\circ\text{C}$ with step of $5 \, ^\circ\text{C}$, drastically increase in power density from 8.888 , 10.465 to $11.136 \, \text{mW m}^{-2}$ at 25 , 30 , and $35 \, ^\circ\text{C}$, respectively, shown in Figure 60 but current density was only effective at $25 \, ^\circ\text{C}$ with a maximum value of $257.649 \, \text{mA m}^{-2}$ while it was $247.536 \, \text{mA m}^{-2}$ for the next most current density formed at $35 \, ^\circ\text{C}$. An increase in current and power densities at this temperature which were resulted due to increase in metabolic and enzymatic activity of *E. coli* cells. Temperatures beyond $40 \, ^\circ\text{C}$ might damage important ingredients such as proteins, nucleic acids, and others temperature-sensitive components irreversibly as described by other study [247], which would lead to reversing the curve and fall in performance. It was probably the main reason of decrease in maximum power output when temperature became higher than $40 \, ^\circ\text{C}$. After $40 \, ^\circ\text{C}$ and elevated temperature *E. coli* growth becomes suppressed and decrease power conversion efficiency. Therefore, maximum power density in the temperature between 25 - $35 \, ^\circ\text{C}$ was effective for *E. coli* which is very close to average temperature of $28 \pm 2 \, ^\circ\text{C}$.

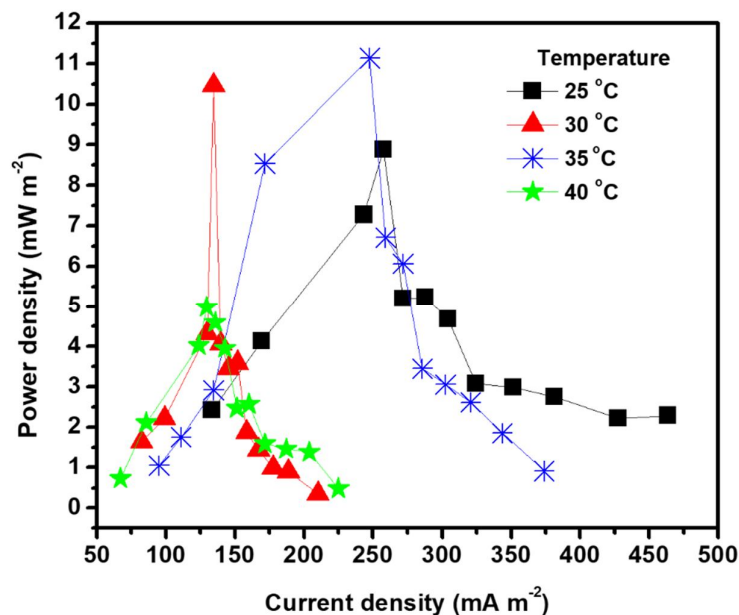


Figure 60. Power density as a function of current density using *E. coli*, $R = 500 \Omega$, $\text{pH} = 7.42$, glucose = 100 mg L^{-1} .

The second way of optimizing the cell was operating MFC without connecting with external resistance known as steady state. At this operation bare PGE, $\alpha\text{-MnO}_2/\text{PGE}$, PANI/PGE , $\alpha\text{-MnO}_2/\text{PANI}/\text{PGE}$, and $\alpha\text{-MnO}_2/\text{NiO}/\text{PANI}/\text{PGE}$ were selected as a test anode due to having the better electrical conductivity of PANI/PGE , $\alpha\text{-MnO}_2/\text{PANI}/\text{PGE}$, and $\alpha\text{-MnO}_2/\text{NiO}/\text{PANI}/\text{PGE}$ than NiO/PGE and $\alpha\text{-MnO}_2/\text{NiO}/\text{PGE}$ while bare PGE was used as a reference and $\alpha\text{-MnO}_2/\text{PGE}$ was a basic catalyst in this study. So, at steady state open circuit voltage (V_{OC}) is one of the most important criteria to investigate the catalytic activity of the prepared materials for better MFC performance.

Thus, V_{OC} responses obtained by $\alpha\text{-MnO}_2$, PANI , $\alpha\text{-MnO}_2/\text{PANI}$, $\alpha\text{-MnO}_2/\text{NiO}/\text{PANI}$ modified PGE and bare PGE were operated for four continuous days each with a total of 20 days (Figure 61a). In each day a continuous 3 hour measurement per day was operated. Throughout operations, the maximum V_{OC} of $471.17 \pm 89.82 \text{ mV}$ (day 1), $348.58 \pm 42.04 \text{ mV}$ (day 2), $616.72 \pm 56.65 \text{ mV}$ (day 2), $650.61 \pm 10.11 \text{ mV}$ (day 3), $222.36 \pm 8.16 \text{ mV}$ (day 4) were produced by PANI/PGE , $\alpha\text{-MnO}_2/\text{PGE}$, $\alpha\text{-MnO}_2/\text{NiO}/\text{PANI}/\text{PGE}$, $\alpha\text{-MnO}_2/\text{PANI}/\text{PGE}$, and PGE, respectively. The selected time is considered as the best time to operate MFC at steady state. From this investigation, highest V_{OC} was recorded by using $\alpha\text{-MnO}_2/\text{PANI}/\text{PGE}$ and $\alpha\text{-MnO}_2/\text{NiO}/\text{PANI}/\text{PGE}$ was the next anode during the third and second day operating time. This increment is due to the best effective electron transfer

medium between electrogenic *E. coli* with PGE resulting from the effective electrical conductivity found in α -MnO₂/PANI/PGE and α -MnO₂/NiO/PANI/PGE which was proved by EIS.

Not only this due to the effective surface roughness nature found on these two PANI based composites, they have a capability to adsorb more *E. coli* on their surface which is responsible to form a conductive biofilm with rises in V_{OC} as shown in Figure 61b. This maintained that V_{OC} after 3 days of operation was found to be higher with α -MnO₂/PANI hybrid composite modified PGE that leads to improve fast electron transfer rate on anode surface in MFC. So, this is an indication to enhance MFC performance using these composites due to having effective electroactive surface area which was proved by *Randles-Sevcik* equation from CV. To clarify more, Table 22 compares the V_{OC} produced by five anodic electrodes under relatively identical conditions. The unusually high V_{OC} may be attributed to the high charge density capacity on the modified electrode which was previously proved by CV measurements and this also increases subsequently due to conductive biofilm establishment after interacting with *E. coli* by forming bioanode. Katuri et al. [251] justified that when EAB is collected and attached near or on non-toxic conductive electrodes, they be able to interact with each other without damaging its cellular respiration. This interactions cause to increase conductive biofilm formation by increasing its surface active area on the electrode surfaces which leads to improved fast electron transfer rate on anode surface in the MFC operation. It is clear that the smaller α -MnO₂ NPs integration in PANI conductive matrix created a good synergetic effect than pure PANI and α -MnO₂ NPs resulted for stabilized nanocomposite catalyst formation in the MFC. So, it is concluded that the fabricated nanocomposite could be suggested as a good and effective bioanode material to increase MFC performance towards bioremediation and bioelectricity.

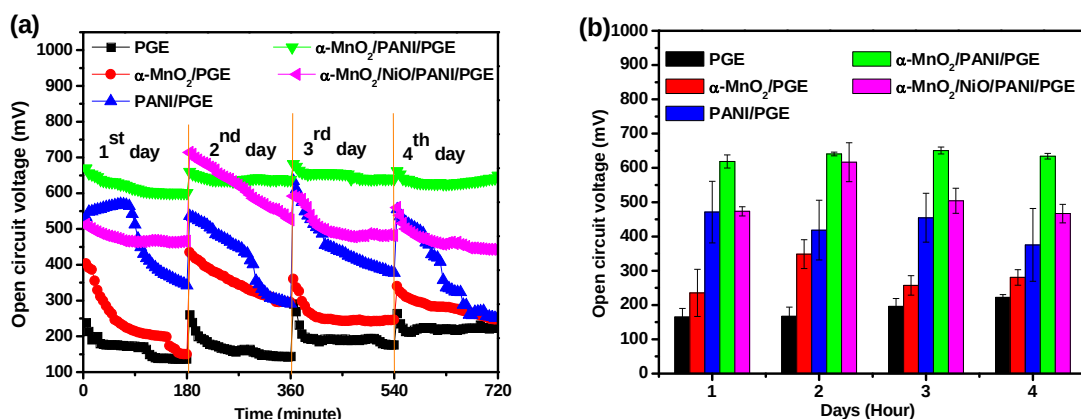


Figure 61. (a) Variation of open circuit voltage and (b) variation of average open circuit voltage with time.

Table 22. Comparison of V_{OC} output for five bioanodes.

Time of operation	Electrode	Mean $\pm$ SD (mV)
1 st day	PGE	164.98 $\pm$ 24.84
	α -MnO ₂ /PGE	235.36 $\pm$ 69.03
	PANI/PGE	471.17 $\pm$ 89.82
	α -MnO ₂ /PANI/PGE	618.78 $\pm$ 19.09
	α -MnO ₂ /NiO/PANI/PGE	473.39 $\pm$ 13.29
2 nd day	PGE	166.92 $\pm$ 27.03
	α -MnO ₂ /PGE	348.58 $\pm$ 42.04
	PANI/PGE	418.61 $\pm$ 87.35
	α -MnO ₂ /PANI/PGE	640.42 $\pm$ 5.48
	α -MnO ₂ /NiO/PANI/PGE	616.72 $\pm$ 56.65
3 rd day	PGE	195.83 $\pm$ 22.92
	α -MnO ₂ /PGE	257.14 $\pm$ 28.54
	PANI/PGE	454.53 $\pm$ 71.37
	α -MnO ₂ /PANI/PGE	650.61 $\pm$ 10.11
	α -MnO ₂ /NiO/PANI/PGE	504.25 $\pm$ 36.61
4 th day	PGE	222.36 $\pm$ 8.16
	α -MnO ₂ /PGE	280.47 $\pm$ 22.49
	PANI/PGE	375.36 $\pm$ 106.12
	α -MnO ₂ /PANI/PGE	634.00 $\pm$ 8.15
	α -MnO ₂ /NiO/PANI/PGE	466.92 $\pm$ 27.03

4.2.2. Polarization curve and power density

Both of current and power densities are the most important parameter to characterize MFC performance in the bioelectrochemical system based remediation technique [252]. The second ways of optimizing best electrode efficiency was done at non-steady state conditions (*i.e.*, by changing external load) at fixed temperature and pH. Hence to identify the best catalyst modified electrode, tests were done by using glucose (166 mg L⁻¹) as a fuel in the

presence of *E. coli*. First operations were done to see the efficiency of α -MnO₂/NiO bimetallic oxide nanocatalyst modified PGE as compared to α -MnO₂/PGE, NiO/PGE, and bare PGE shown in Figure 62. A maximum V_{OC} of 415 mV with highest power density of $412.24 \pm 5.98 \text{ mW m}^{-2}$ than PGE (269 mV, $65.74 \pm 0.96 \text{ mW m}^{-2}$), α -MnO₂/PGE (336 mV, $315.62 \pm 4.87 \text{ mW m}^{-2}$), and NiO/PGE (328 mV, $140.35 \pm 1.68 \text{ mW m}^{-2}$) was recorded. It can be clearly seen that the performance of MFC with bare PGE bioanode was lowest due to the low biofilm formation on its surface and leads to minimum effective electron transfer efficiency. As a result substrate degradation efficiency become too low. So, to enhance these, the integration of α -MnO₂ and NiO NPs plays a crucial role to improve the electrochemical performance of conventional bare PGE by forming a composite anode. This is the fact that the lower in internal resistance characteristics in the neutral medium in terms of impedance magnitude found on the bimetallic oxide NPs modified PGE increases the electron transfer rate between biofilm and conventional PGE by reducing PGE ohmic resistance. But when measurements were extended with α -MnO₂/PANI/PGE and α -MnO₂/NiO/PANI/PGE, their output V_{OC} and power density were increasing as shown in Figure 62a and b and Table 23. Even, the power density by PANI/PGE with a value of $422.79 \pm 21.77 \text{ mW m}^{-2}$ is significantly higher than α -MnO₂/NiO/PGE due to having lower internal solution resistance and better electrical conductivity nature evidenced from EIS.

Besides electrical conductivity, the swelling and shrinkage behavior of PANI affects the physical strength of bioanode in MFC under neutral medium of conditions while α -MnO₂ NPs have a significant achievement to increase the specific capacitance and cycling stability of conventional carbon electrodes by forming composites under near and neutral solutions [253]. Thus to solve this problems, making composite with α -MnO₂ helps to provide increasing in active surface sites during the polymerization growth of PANI. The presence of active sites was proved by surface roughness and pore size distribution analysis which would help to increase the surface areas of anode; hence integrating α -MnO₂ in PANI matrix improves electrode activity by minimizing barrier of charge transfer and Ohmic resistances. Such minimization increases electron transfer rate during glucose decomposition by *E. coli* metabolism. Conductive composites with rough surface help to attach more *E. coli* on its surface and create a conductive biofilm known as bioanode. Such interactions promoted to produce a higher power output to $426.26 \pm 38.89 \text{ mW m}^{-2}$ and $506.96 \pm 7.31 \text{ mW m}^{-2}$ using α -MnO₂/PANI and α -MnO₂/NiO/PANI composite modified PGE, respectively. The good electron transfer between π orbitals of PANI and d orbitals of Mn⁴⁺-Ni²⁺, enable α -

MnO₂/NiO/PANI composite be more effective in power density than α -MnO₂/PANI composite during glucose oxidation in the given time of operations as shown in Figure 62b.

It is reported that an electrogenic bacteria can transfer electrons produced intracellularly by their central metabolism to an extracellular anode which serves as electron acceptor *via* either self-secreted electron mediators, direct cell membrane-anode coupling, or electrically conducting pili [254]. Once when these electrons arrive at nanocomposite modified PGE then it elevated to the higher potential cathode through an external circuit, forming a current. This is the general basic principle for current generation in a MFC.

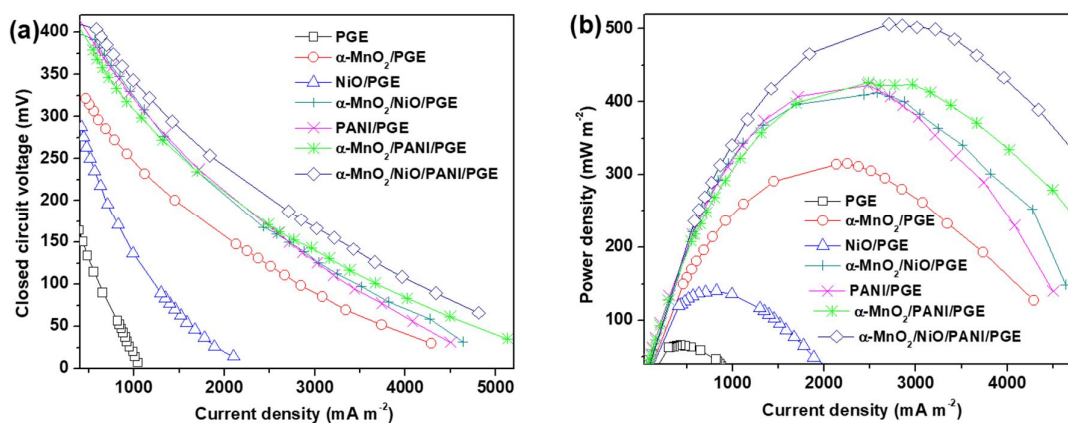


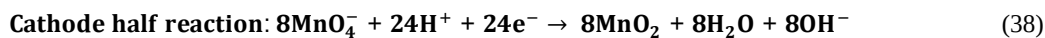
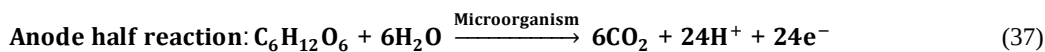
Figure 62. (a) Polarization and (b) Power density curves for different catalyst modified PGE.

Table 23. Summary of optimum power density, current density, open circuit voltage, stabilized time and resistance.

Electrode	Current density at maximum power (mA m ⁻²)	Maximum power density (mW m ⁻²)	Time (Minutes)	External resistance (Ω)	Internal resistance (Ω)	V _{OC} (mV)
PGE	436.52	65.74 ± 0.96	210	5000	2566	269
α -MnO ₂ /PGE	2254.43	315.62 ± 4.87	135	900	801	336
PANI/PGE	2475.36	422.79 ± 21.77	150	1000	1070	459
α -MnO ₂ /PANI/PGE	2485.51	426.26 ± 38.89	150	1000	936	441
α -MnO ₂ /NiO/PANI/PGE	2710.51	506.96 ± 7.31	180	1000	814	427

Note that all parameters except power density are given from average mean values after triplicate measurements, internal resistance and V_{OC} were determined from the fit linear analysis of polarization curves using OriginPro 8 software.

The proposed electrochemical reaction mechanism between catalyst modified anode/anolyte and cathode/catholyte interface towards glucose oxidation is found in the following reactions. This equation is supported by the literature if KMnO₄ is considered as a terminal electron acceptor at cathode chamber [255].



The cation generated from the anode bioreactor during glucose metabolism to carbon dioxide is passed through salt bridge to balance the produced charge in the cathode compartment as it clearly described from the schematic diagram in Figure 63.

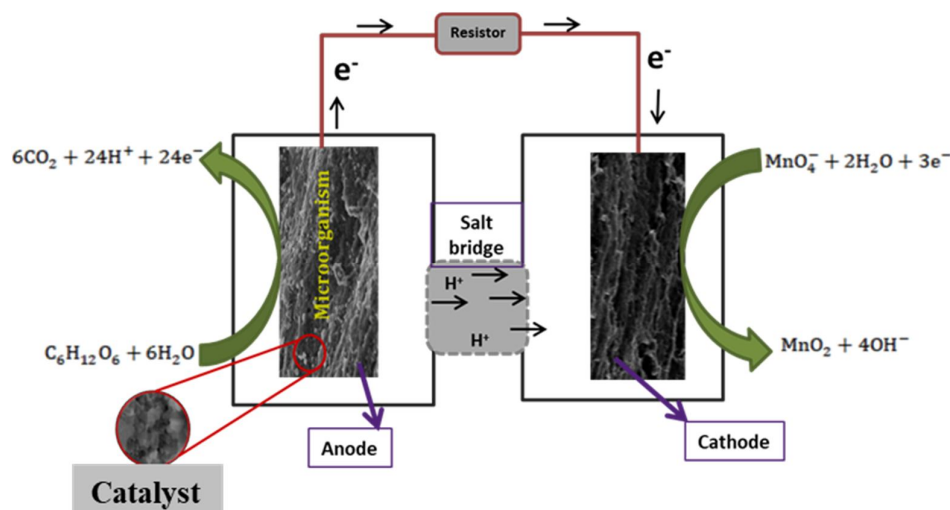


Figure 63. Typical electrochemical catalytic activity and electron transfer mechanism for glucose decomposition in the presence of catalyst modified PGE.

It is clear that the transfer of electrons between π orbitals of PANI, d orbitals of Mn^{4+} , and Mn^{4+} - Ni^{2+} and stimulated the better electrical conductivity in the α - MnO_2 /PANI and α - MnO_2 /NiO/PANI composites elucidated from admittance data, which enable to increase glucose substrate oxidation during MFC operation. So, during glucose oxidation the produced power density was higher in α - MnO_2 /PANI/PGE next to α - MnO_2 /NiO/PANI/PGE as shown in Figure 64a. Again, the higher electrical conductivity and presence of rich pore surface site helps to oxidize glucose using higher external load shown in Figure 64b. A strong bond creation between PGE layer and PANI backbone *via* π - π stacking, electrostatic interactions from carbon material embedded on conducting polymers[256] followed by fast electron transfer between π -d orbitals forms an excellent conduction channels [257,258]. The highest V_{OC} found on PANI/PGE and efficient charge storing capability found on α - MnO_2 and NiO NPs makes α - MnO_2 /NiO/PANI/PGE become efficient in power output at lower external resistance. As time is going on output power density across all electrodes

decreases due to decrease in charge storing capability resulting from the decrease in life cycle stability as shown in Table 23. The free flow of charge in the MFC always hindered as a result of the presence of anodic resistance [28]. Increasing the conductivity of anode materials could be able to minimize contact resistance, Ohmic losses, and slow electron transfer rate. As time is goes to higher external resistance their effective output power is decreasing due to the continuous charge loss from glucose concentration reduction.

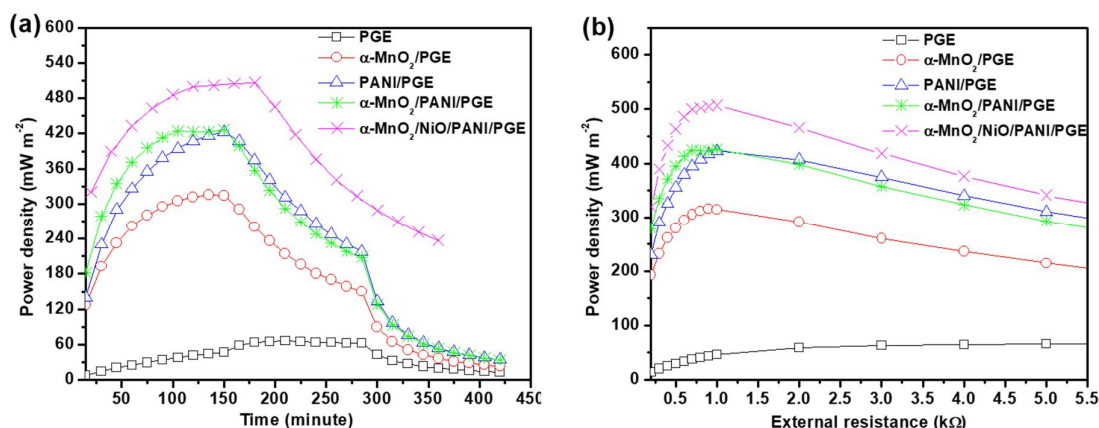


Figure 64. Dependence of power density on time (a) and external resistance (b).

Thus, the maximum current discharge observed using $\alpha\text{-MnO}_2/\text{PANI/PGE}$ and $\alpha\text{-MnO}_2/\text{NiO/PANI/PGE}$ was 0.1715 mA and 0.1870 mA, respectively, at 1000 Ω while the current discharge observed with bare PGE is 0.0301 mA at 5000 Ω . An increase in surface areas lowers the internal resistance found on the modified electrodes indicated fast extracellular electron transfer between the *E. coli* and bioanode. The flow of free charge hindered using bare PGE might arises due to its highest internal resistance found from the polarization curve. Thus the calculated internal resistance obtained from slopes of measured voltage *versus* current density [259] were given and low in composites modified PGE than bare PGE and the optimum non-steady state process parameters are summarized in Table 23.

4.2.3. Organic matter removal and coulombic efficiencies

In addition to studying power generating, biodegradation of glucose based artificial wastewater was done. An organic waste liquid matter removal in terms of concentration change and removal efficiency (COD %) during V_{OC} measurements by using different catalyst modified PGE and bare PGE were shown in Figure 65a and b. After 4 days with 3 hours per day measurement, a concentration change of $59.05 \pm 1.11 \text{ mg L}^{-1}$, $34.69 \pm 0.60 \text{ mg L}^{-1}$, $33.23 \pm 0.27 \text{ mg L}^{-1}$, $12.84 \pm 0.29 \text{ mg L}^{-1}$, $38.73 \pm 2.17 \text{ mg L}^{-1}$ were recorded using

PGE, α -MnO₂/PGE, PANI/PGE, α -MnO₂/PANI/PGE, and α -MnO₂/NiO/PANI/PGE, respectively, (see Appendix, Table A1). The lower concentration removal with best COD of 92.27% was obtained by α -MnO₂/PANI/PGE due to the better integration between α -MnO₂ and PANI, results for active biofilm formation that enhance the fast anaerobic respiration due to attaching more *E. coli* due to having high roughness surface. The image of the obtained biofilm on anodic surface is clearly showed the the adhesion of *E. coli* microorganism on the surface of α -MnO₂/PANI composite modified PGE which were much effective than other. This indicated that the fast electron transfer across these composite facilitates glucose oxidation which insured it's excellent in removal efficiency. Karthikeyan et al. [260] have reported PANI modified chitosan/titanium carbide composites anode electrode with COD of 87-93%. A slight higher in glucose concentration change with COD under V_{OC} and CCV obtained by α -MnO₂/PANI/PGE compared with other bioanode catalysts might be due to the enhanced glucose oxidation caused by highly enriched electroactive *E. coli* on its surface because of its improved surface roughness structure and electrochemical properties.

During CCV measurements (*i.e.*, non-steady state conditions) for 420 minutes, an average concentration removal of $54.84 \pm 6.54 \text{ mg L}^{-1}$, $41.42 \pm 0.98 \text{ mg L}^{-1}$, $35.08 \pm 3.67 \text{ mg L}^{-1}$, $19.62 \pm 0.92 \text{ mg L}^{-1}$, and $30.02 \pm 1.77 \text{ mg L}^{-1}$ were recorded using PGE, α -MnO₂/PGE, PANI/PGE, and α -MnO₂/PANI/PGE, and α -MnO₂/NiO/PANI/PGE, respectively, (see Appendix, Table A2). Maximum COD and CE efficiencies of 88.19% and 26.09%, respectively, were obtained from α -MnO₂/PANI modified PGE bioanode, whereas PANI modified PGE anode delivered a CE of 14.54% this value is even higher than α -MnO₂/NiO/PANI as shown in Figure 65b. But the COD of α -MnO₂/NiO/PANI was 81.92% which has better glucose substrate oxidation than PANI/PGE with a value of 78.89%. The PGE electrode without any catalyst modification but under inoculum pretreatment showed a maximum CE of 2.29% only. COD of bare PGE was 67.0% which is too low due to the slower glucose biodegradation rate over a given time period. Thus the transfer of electron from glucose oxidation to bare anode is so slow. The highest CE obtained in α -MnO₂/PANI/PGE followed by PANI/PGE electrodes showed that pretreatment of PGE with *E. coli* in the presence of α -MnO₂/PANI and PANI catalysts reduces the charge transfer resistance and enhanced electron recovery from glucose substrate as evidenced from their maximum V_{OC}.

A 14.54% increase in CE was observed in PANI modified PGE electrode compared with α -MnO₂/NiO/PANI/PGE, α -MnO₂/PGE, and bare PGE, due to enhanced biocompatibility of the PANI modification which increases the charge recovery from glucose and resulted in an improvement in CE. On the other hand, the Mn⁴⁺ and some other metallic oxide based nanocomposite enhances electron transfer rate between the microorganisms and the anode material which facilitates electron conduction but its biocompatibility nature is not effective as PANI [261]. The other reason for effectiveness with CE might happened when a peptide bond formation between PANI, *E. coli*, and PGE surface which serve as a reach to transfer electron rate effectively [129]. So, to support this study further, an increase in CE from 17% to 42.45% was done by carbon felt and PANI modified carbon felt anode, respectively, under dual chamber MFC operation [262].

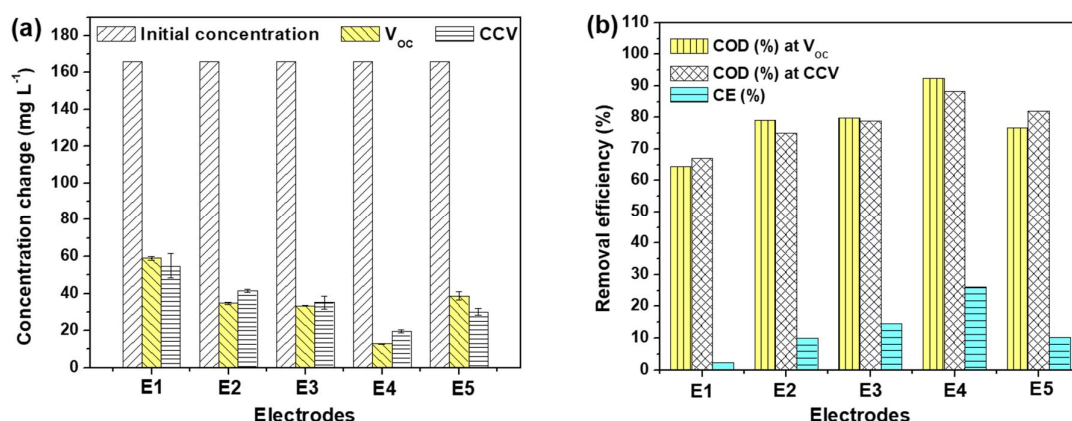


Figure 65. (a) Concentration change and (b) removal efficiency for E1(PGE), E2(α -MnO₂/PGE), E3(PANI/PGE), E4(α -MnO₂/PANI/PGE), and E5(α -MnO₂/NiO/PANI/PGE) bioanode.

Literature survey indicates that, electrons which are a source of energy generated from organic compound can either be used for biosynthesis of cellular compounds, such as proteins or enzymes, lipids, DNA, sugars, and so forth or to create energy carrier molecules, such as ATP and NADH. It is the formation of latter compounds that require electrons be transferred down an electrochemical gradient within the cell to a terminal electron acceptor (*i.e.*, ATP synthesis *via* the electron transport chain). In MFCs and other bioelectrochemical cells, the terminal electron acceptor is the anode. The fractions of electrons that don't flow directly to anode are directly participated in the biosynthesis process. Therefore, the lower CE values typically indicate that the exoelectrogens within the biofilm are using more of

the energy extracted from the substrate for biosynthesis, not energy production, whereas higher CE would indicate the opposite [46].

4.2.4. Biofilm characterization

After MFC operation, biofilm formations on bioanode were studied by SEM imaging. The surface of bare PGE before *E. coli* attachment shows porous topographic view, as shown in Figure 66a. It is evidenced that, the roughness of the electrodes is related to the granularity of the conductive materials [263]. After inserting in an anodic chamber containing colonies of *E. coli*, it is easily observed that portions of *E. coli* cells are adhered to PGE (Figure 66b) due to its hydrophilicity nature. However, due to the poor charge storing capacitive and little biocatalytic activity of carbon electrode materials in MFC performance [39], PGE was successfully modified with α -MnO₂ and α -MnO₂/NiO integrated PANI nanocomposites as shown in Figure 66c and d. From this PANI/PGE anode exhibits a higher bacterial colony loading than α -MnO₂/PGE, which can be attributed to the positively charged PANI backbone that facilitates biocompatibility and its electrostatic interaction with negatively charged *E. coli* outer cell membrane.

It is interesting that after integration of α -MnO₂ and α -MnO₂/NiO in PANI, it is noted that *E. coli* community growth on α -MnO₂/PANI/PGE and α -MnO₂/NiO/PANI/PGE surface to form a thick biofilm (Figure 66e and f) due to the effective *E. coli* loading rate. All this modifications has a positive result in the direct electron transfer improvement between microbe outer membrane c-type cytochromes of *E. coli* and composites and such growth to the surface of the electrode helps to facilitate electrochemical reactions (*i.e.*, substrate degradation and dye decolorization). In all cases the involvements of PANI in α -MnO₂ and α -MnO₂/NiO possess high electron mobility, stability, biocompatibility, anticorrosion nature, excellent electrokinetics which improves the anode performance *via* enhanced microbial growth. Such modification provided more area of the biofilm growth and interaction of the EAB than the unmodified bare anode electrode [25]. So, from such architecture PANI based metallic oxide aided PGE anode surface provide a large surface area for enhanced electron collection from the fast glucose oxidation during *E. coli* respiration, indicated the best anode than other electrodes. Therefore, among electrodes α -MnO₂/PANI/PGE the favorable electrode for biofilm growth results for improved MFC performance towards better power production and wastewater treatment efficiency as evidenced from polarization measurements.

Xu et al. [40] have reported that when a bare carbon electrode is modified by conductive composites from PANI increases its surface roughness and biocompatibility, which is more comfortable to attach more biofilms that helps to become more conducive [40]. So, based on these prospective, fabricated composite materials are beneficial to promote interactions between *E. coli* and anodes for fast extracellular electron transfers activity for better MFC performance operation.

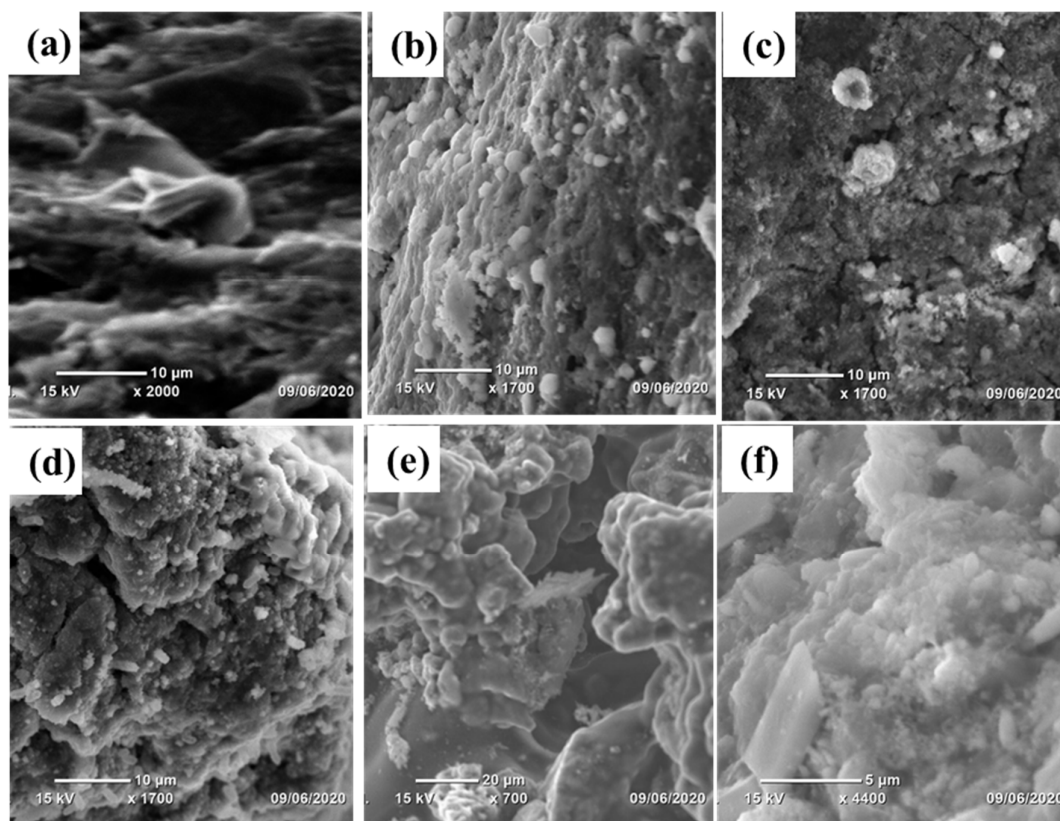


Figure 66. SEM images of (a) bare PGE without *E. coli* and (b) PGE, (c) α -MnO₂/PGE, (d) PANI/PGE, (e) α -MnO₂/PANI/PGE anodes, and (f) α -MnO₂/NiO/PANI/PGE with *E. coli* cells on.

4.2.5. Methyl red removal and kinetic analysis in MFC

The decolorization application of MR was done in double chambered MFC at 200 Ω -10 k Ω external resistance and temperature 28 ± 2 °C. The removal performance of MR in MFCs equipped with α -MnO₂/PANI modified PGE is shown in Figure 67. Figure 67a showed that the typical UV-Vis spectra of MR during decolorization (0-6 hour) in MFC- α -MnO₂/PANI modified PGE. The absorption spectrum of the original anode solution is characterized by the main band in the visible region with its maximum absorption at 428 nm and by the band

in the ultraviolet region located at 257 nm. The absorbance peak at 428 nm is attributed to the azo bonds of MR molecule while the absorbance peak in UV region at 257 nm is attributed to aromatic rings ($\pi \rightarrow \pi^*$ transition) [264]. It was indicated that the dye decolorizing image was decreased gradually as shown on the inset image from Figure 67a. This decrease in absorbance peak described that the dye has been degraded. The appearance of an extra absorbance peaks (200-300 nm) after 4 and 6 hours might be absorbance of metabolic by-products or compounds originating from dye degradation. This indicates that biodegradation in the presence of *E. coli* biocatalyst forms a biofilm on the surface of the composite modified anode contributed as an active metabolite agent for MR decolorization mechanism. The statement has been also supported by other workers. They explained that, biodecolorization that occurs as a result of biodegradation is characterized by either the disappearance of a major absorbance peak or the appearance of a new peak and the red color of MR cultured medium consequently turned to colorless due to the reductive breakdown of azo (C–N=N–C) bond, while the presence of new peak was probably due to the production of metabolites as an intermediates [42].

The COD removal performance during MR decolorization at the same MFC reactor was achieved using PGE and α -MnO₂/PANI/PGE shown in Figure 67b. The COD removal rate using α -MnO₂/PANI/PGE in the presence of MR was better than that of unmodified PGE during the course of the reaction. The removal rate was increases with increasing reaction time, almost $74.59 \pm 1.57\%$ of COD was achieved within 6 hours reaction time for MFC- α -MnO₂/PANI/PGE, while $67.29 \pm 0.63\%$ for MFC-unmodified PGE (Table 24). The better performance of MR removal and electricity generation in MFC- α -MnO₂/PANI/PGE might be the better biocompatibility and charge transfer capacity nature of α -MnO₂/PANI composite. At the anode chamber of a MFC, glucose is oxidized by electrochemically active *E. coli*, followed by fast transfer of generated electrons to the anode with the help of nanocatalysts which then passes through an external circuit to the cathode, thus producing current. When MR dye is added into the anode chamber, the reductive cleavage of azo bond found in the structure of azo dye would consume partly the electrons from substrate oxidation [265,266]. So, the decolorization mechanism of MR and power output using α -MnO₂/PANI deduce more electrons from glucose substrate to the modified anode surface. As a consequence, process in the presence of MR could consume more electrons by the added dye, resulting in a better decolorization performance.

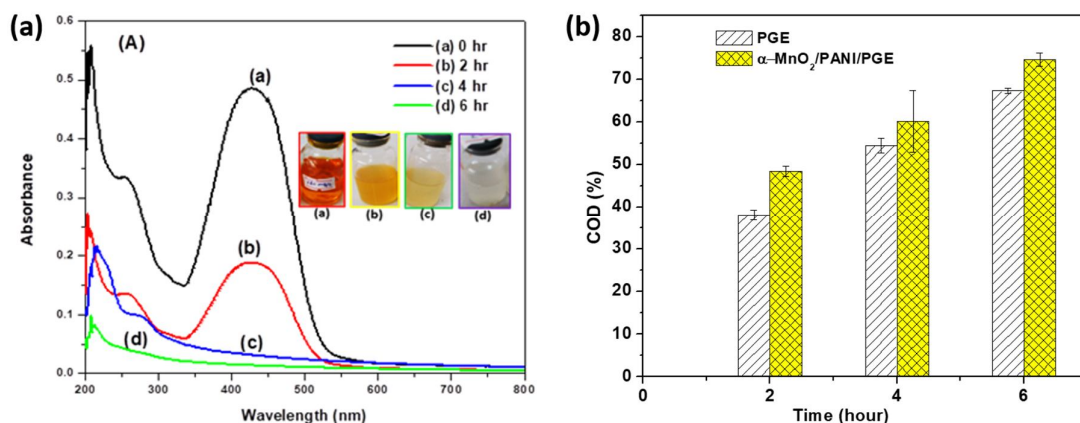


Figure 67. UV-Vis absorption spectra of MR during the decolorization process in MFC using α -MnO₂/PANI/PGE (a) and COD removal rate of 160 mg L⁻¹ MR in MFCs equipped with PGE and α -MnO₂/PANI/PGE anodes (b).

Table 24. Summary of COD removal rate and decolorization efficiency comparison between bare PGE and α -MnO₂/PANI/PGE anode electrodes (substrate = glucose, 160 mg L⁻¹ and MR, 160 mg L⁻¹).

Bare (PGE)			α -MnO ₂ /PANI/PGE	
Time (hour)	COD (%)	Decolorization efficiency	COD (%)	Decolorization efficiency
0	0	0	0	0
2	38.13 ± 1.25	43.03 ± 3.22	48.51 ± 1.21	60.96 ± 0.35
4	54.47 ± 1.67	65.60 ± 6.65	60.11 ± 7.29	90.21 ± 3.00
6	67.29 ± 0.63	85.12 ± 2.86	74.59 ± 1.57	95.57 ± 2.26

A_0 is the absorbance of the initial while A_t is the absorbance of solution taken from the anode chamber at a certain reaction time t (hour); absorbance was recorded at $\lambda_{\max} = 428$ nm.

As shown in Figure 68a, the decolorization efficiencies of MR using bare PGE and MnO₂/PANI/PGE were 43.03 ± 3.22% and 60.96 ± 0.35%, respectively, after 2 hour running. As the reaction time become 6 hours, the decolorization efficiencies had reached to 85.12 ± 2.86% and 95.57 ± 2.26%, respectively. The good decolorization effect by α -MnO₂/PANI/PGE during the time of operation as shown in Table 25 indicated that PGE coated by MnO₂/PANI nanocomposite had good decolorization efficiency with in a limited time due to the better porous structure which provides more contact for the adhesion of conductive biofilms. Besides, the good electron transfer efficiency of α -MnO₂ near neutral media and biocompatibility of PANI promoted the growth of microbes and accelerated glucose and MR inside its pore, thereby facilitating glucose oxidation and decolorization. Thus, the decolorization efficiency using α -MnO₂/PANI/PGE was higher than that of bare

PGE, which demonstrated that decolorization performances could be further improved after modification.

Moreover, kinetics of biodegradation of reactive MR was studied using semi-log MR concentration and time relationship shown in Figure 68b. It demonstrates that the reaction kinetics of MR decolorization followed first order kinetic model with Adjusted R-Square (R^2) of 0.98331 and 0.95484 for PGE and α -MnO₂/PANI/PGE, respectively. That is, the color degradation efficiency using modified (α -MnO₂/PANI/PGE, first order rate constant 0.21827 hour⁻¹ close to 4.58 hours) is formed in a faster rate than unmodified (PGE, first order rate constant 0.18296 hour⁻¹ close to 5.50 hours) anode (see Table 25). Generally, modification of carbon materials due to having little electrocatalytic activity with α -MnO₂ NPs accelerates the extracellular electron transfer rates from electrogenic microbes to the anode due to its high capacitive behavior, which helps to improve MFC performance by increasing the cycle stability of anode [267]. On the other hand, the high bioelectrocatalytic activity of PANI, which made it a better extracellular electron transport capacitor compared with the unmodified anode [39]. Consequently, when PGE is modified with α -MnO₂/PANI composite, the anode could rapidly transfer a large amount of electrons from microbe metabolism to electrode surface and extracellular MR dye molecules through the electron transfer chain. Thus, the high efficient color degradation of extracellular MR would be achieved with a good power output shown on the polarization graphs.

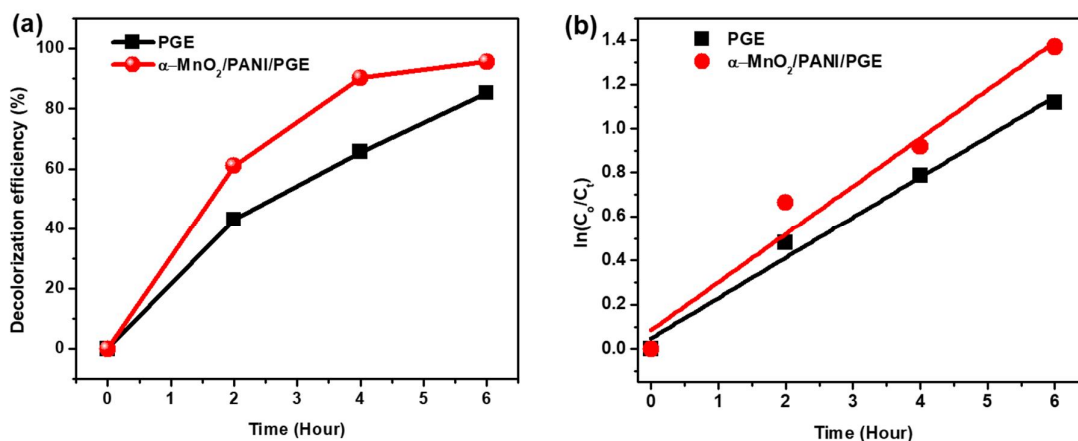


Figure 68. Decolorization efficiency (a) and fitting first order kinetic curves for MFCs with bare PGE and α -MnO₂/PANI/PGE composite anodes (b).

Table 25. First order kinetic parameters and their ANOVA analysis.

Anode	Equation	Rate constant (hour ⁻¹)	Adj. R-Square (R ²)	Probability
PGE	Y = 0.18296X + 0.04724	0.18296	0.98331	<0.00558
α -MnO ₂ /PANI/PGE	Y = 0.21827X + 0.08341	0.21827	0.95484	<0.01517

Note: Statistical analyses were done by OriginPro 8 software. $\ln \frac{C_0}{C_t} = Y$; t = X, and slope = rate

constant (k); $\ln \frac{C_0}{C_t} = kt$ first order

4.2.6. Methyl red decolorization and electricity generation

The polarization and power density curves of α -MnO₂/PANI modified and bare PGE are shown in Figure 69a. The MFC with α -MnO₂/PANI/PGE based anode in the absence of MR dye have lower V_{OC} (396.59 mV) and lower internal resistance (1189.05 Ω) than anode in the presence of MR dye with V_{OC} (666.17 mV) and 2287.88 Ω of internal resistance. An increase in internal resistance during decolorization of MR dye might come from anolyte solution resistance during non-steady state conditions. The total internal resistance formed in each anodic electrodes were calculated from the slope of voltage *versus* current fitted curve plot [166]. On the other hand, the MFC with bare PGE exhibited the higher slope (3745.74 Ω), indicating that the polarization losses is higher due to the slow extracellular electron transfer rate which has higher internal resistance than MR- α -MnO₂/PANI/PGE as shown in Table 26.

The power density curve shown in Figure 69b demonstrated that the maximum power density of the MFC with the MR- α -MnO₂/PANI/PGE was increased by 91.98%, 58.12%, and 48.02% than PGE, MR-PGE, α -MnO₂/PANI/PGE, respectively, to 820.02 ± 7.86 mW m⁻² with I_{max} of 1990.34 mA m⁻² (see Table 26). PGE modified with α -MnO₂/PANI composite had more bioelectrocatalytic active sites to facilitate all bioelectrochemical reactions on its surface; especially, the addition of high capacitance α -MnO₂ with good biocompatible PANI would cause more *E. coli* colonies to adhere and attach on the electrode to form a biofilm. Thus, the formed biofilm found to promote the electron transfer rate between *E. coli* and anode in the MFC. Therefore, due to the modification of PGE from the high bioelectrocatalytic activity of PANI and good charge capacitive behavior of α -MnO₂, current generation of the MFC constructed with α -MnO₂/PANI/PGE in the presence of MR dye was significantly improved. The presence of MR dye during its decolorization produces high current output using α -MnO₂/PANI/PGE. The decolorized intermediates after decolorization of dyes might act as an electron-shuttling mediator to enhance bioelectricity

generation in the MFC [268,269]. Especially, amine-based dye could act as good artificial indirect electron transfer mediator [269]. So, α -MnO₂/PANI/PGE improved the simultaneous pollutant removal and electricity production enhancement, which could help a simultaneous bioenergy recovery and reduce processing costs during wastewater treatment process.

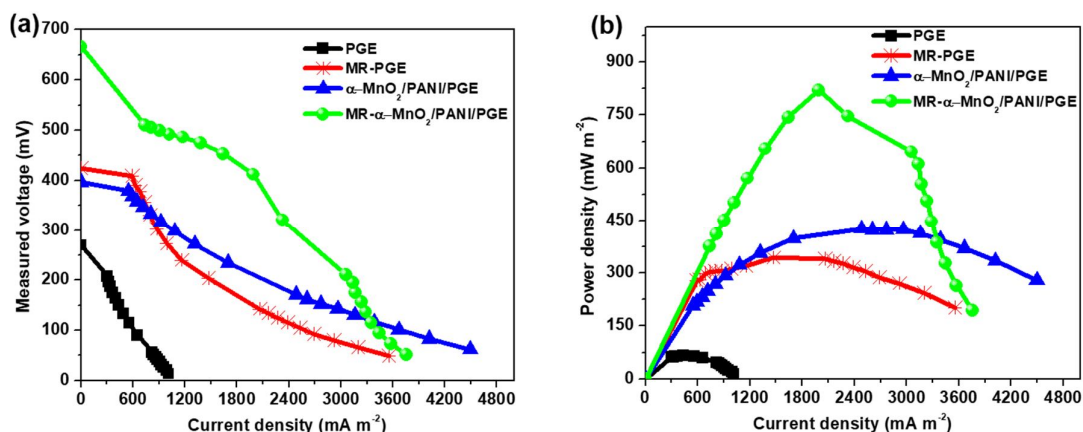


Figure 69. Polarization curves (a) and power density curves of MFCs (b) with different anodes in the presence and absence of MR.

Table 26. Polarization parameters and V_{OC} at steady state conditions of the MFC.

Anode	P_{max} (mW m ⁻²)	I_{max} (mA m ⁻²)	Internal resistance (Ω)	CCV (mV)	V_{OC} (mV)	Adj. R- Square (R^2)
PGE	65.74	436.52	3745.74	150.6	269.93	0.98858
MR-PGE	343.41	1471.01	1762.10	203.0	424.91	0.92426
α -MnO ₂ /PANI/PGE	426.26	2485.51	1189.05	171.5	396.59	0.97349
MR- α -MnO ₂ /PANI/PGE	820.02	1990.34	2287.88	412.0	666.17	0.97153

Glucose concentration = 160 mg L⁻¹, MR concentration = 160 mg L⁻¹, Adjusted R-Square is determined from linear fitting statistics of voltage vs current. **Note:** all the graphs and other parameters are determined from mean values.

4.2.7. Effect of methyl red concentration on power generation

The effect of MR dye was investigated by varying its concentration at 50, 100, 150, and 200 mg L⁻¹ at the same glucose concentration of 100 mg L⁻¹. Figure 70a shows that the decolorization efficiency of MR at different concentrations can reach up to 90.11%, indicating that α -MnO₂/PANI/PGE had high decolorization performance efficiency. The rate of decolorization performance at the given time decreased as the concentration of MR increased except at 100 mg L⁻¹. The decolorization efficiency at 50, 150, and 200 mg L⁻¹ for 6 hours were 84.79%, 70.08%, and 60.25%, respectively, while 90.11% decolorization

efficiency was obtained at 100 mg L⁻¹. After 6 hours 6.01, 9.63, 34.15, and 83.79 mg L⁻¹ decolorized MR concentration were obtained as shown in Figure 70b, indicating that the amount of decolorization of MR after MFC treatment were increased gradually with the raised MR concentration. The greater the concentration of the MR dye contributes to produce more electrons by active *E. coli* in the given time.

Provided that dyes are poisonous which might slows down *E. coli* activities that found on the anode chamber of MFC, results for low removal efficiencies except at 100 mg L⁻¹ shown in Figure 70d and Appendix, Table A3. At 100 mg L⁻¹ the removal efficiencies was reached to 90.37% after 6 hours, due to the mutual concentration existence between MR and glucose co-substrate (1:1) which might create a poisonous tolerance for *E. coli*. It can be seen from Figure 70c and Table 27, the reaction kinetics of MR decolorization in the selected concentration clearly followed first order kinetic model, and their rate constants were 0.3485, 0.3875, 0.2310, and 0.1410 hour⁻¹ for 50, 100, 150, and 200 mg L⁻¹, respectively. The model showed that from the selected dye concentration, the rate of MR decolorization and rate of metabolic reactions on α -MnO₂/PANI/PGE in the presence of *E. coli* biocatalyst were fastest and took small reaction time at 100 mg L⁻¹ due to the lower poisonous nature than at 150 mg L⁻¹ and 200 mg L⁻¹, as well as lower electron production by 50 mg L⁻¹.

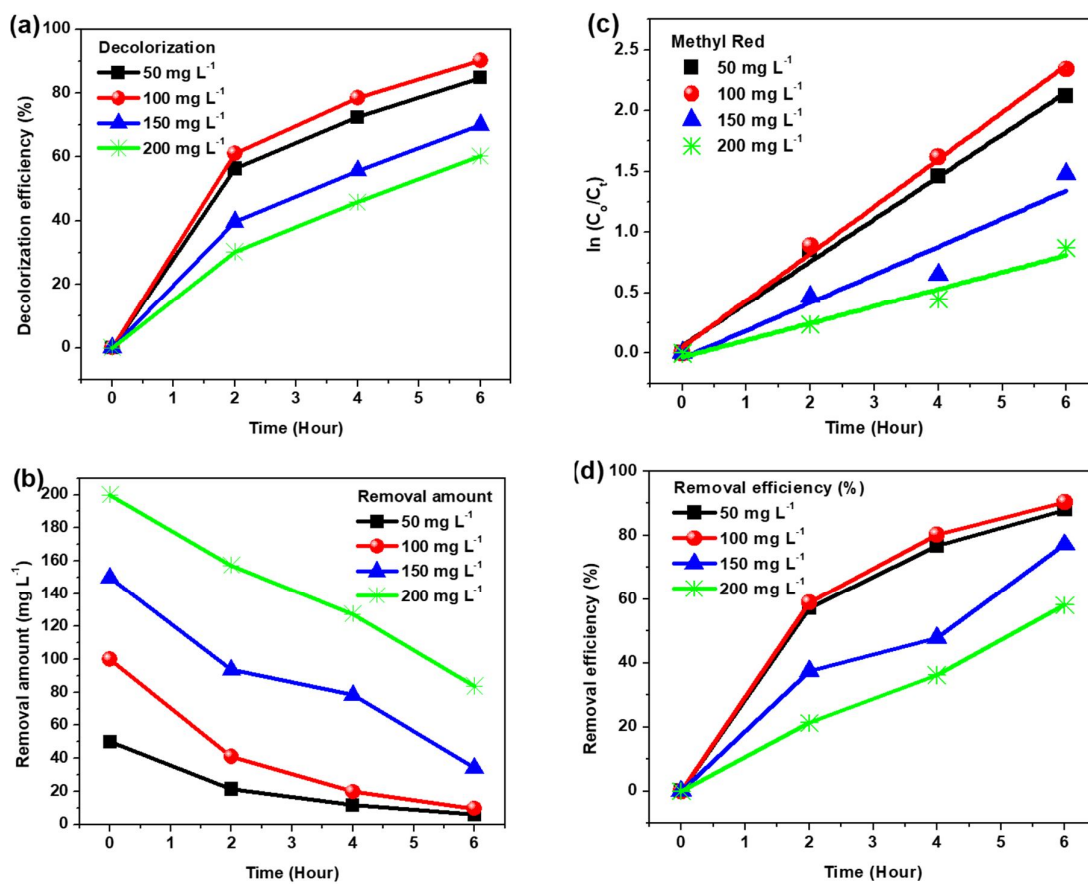


Figure 70. Effect of MR concentration on decolorization efficiency (a), removal amount (b), first order kinetic curve (c), and removal efficiency (d) using α -MnO₂/PANI/PGE.

Table 27. First order kinetic parameters and their ANOVA analysis using α -MnO₂/PANI/PGE (glucose concentration = 100 mg L⁻¹).

MR concentration (mg L ⁻¹)	Equation	Rate constant (hour ⁻¹)	Adj. R-Square (R ²)	Probability
50	$Y = 0.3485X + 0.062$	0.3485	0.99187	0.00271
100	$Y = 0.3875X + 0.05$	0.3875	0.99584	0.00139
150	$Y = 0.231X - 0.043$	0.2310	0.89957	0.03406
200	$Y = 0.141X - 0.033$	0.1410	0.95969	0.01353

Note: Statistical analyses were done by OriginPro 8 software. $\ln \frac{C_0}{C_t} = Y$; $t = X$, and slope = rate constant (k); $\ln \frac{C_0}{C_t} = kt$ is the first order kinetics

The internal resistance of 919.01 Ω at 100 mg L⁻¹ MR concentration determined from measured voltage verses current curve was smallest than other concentrations shown in Figure 71a and Table 28, while the slope of polarization curve found from the rest of MR concentrations were larger. Their internal resistances were 1395.80, 1323.31, and 1292.84 Ω , at 50, 150, and 200 mg L⁻¹ MR concentration, respectively. Their maximum power densities shown in Figure 71b were 422.79 ± 21.77 , 335.28, and 178.24 mW m⁻²,

respectively. Compared with the MFC at 100 mg L⁻¹ MR ($P_{\max} = 735.66 \text{ mW m}^{-2}$), the power generation performance decreased with increasing MR concentration, and the maximum power density at 100 mg L⁻¹ MR was increased by 42.53%, 54.42%, and 75.77% at 50, 150, and 200 mg L⁻¹ MR concentration, respectively. This happened due to the electrons produced by microbial oxidation of co-substrate which had two orientations: one was to generate current by direct or indirect transfer to the anode and the other was obtained by dye molecule to break the $-N=N-$ bond of MR. Therefore, at the same co-substrate concentration, the higher initial MR concentration needs more electrons consumed for reductive decolorization. So, the electronic competition would be more intense, and the output current and power generation capacity of MFC were gradually reduced. In addition, MR with strong toxicity would poison the exoelectrogens activity of *E. coli*, reduce their biological activity, and inhibit the ability to produce and transfer electrons. At the low concentration of methyl orange ($\leq 100 \text{ mg L}^{-1}$), it took little effect on the energy output of MFCs [40].

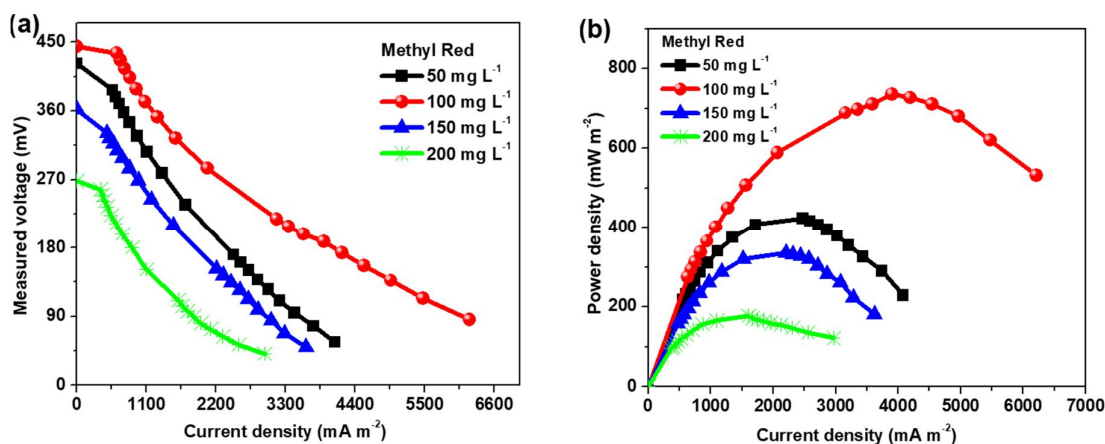


Figure 71. Polarization (a) and power density (b) curves of MFCs in different concentration of MR (glucose = 100 mg L⁻¹) using α -MnO₂/PANI/PGE composite anode.

Table 28. α -MnO₂/PANI/PGE based polarization parameters at different MR concentrations.

MR concentration (mg L ⁻¹)	$P_{\max}$ (mW m ⁻²)	$I_{\max}$ (mA m ⁻²)	Internal resistance (Ω)	CCV (mV)	V_{oc} (mV)	Adj.R-Square (R^2)
50	422.79	2475.36	1395.80	170.8	422.42	0.98632
100	735.66	3902.69	919.01	188.5	444.67	0.96979
150	335.28	2204.35	1323.31	152.1	362.39	0.9925 0
200	178.24	1607.25	1292.84	110.9	268.22	0.95444

Glucose concentration = 100 mg/L, All numerical values are mean values.

The findings of the present work were compared with the already existing results and are summarized in Table 29. It is significant to note that the electricity generation with simultaneous waste treatment (COD removal and decolorization efficiency) suggested in the present work has relatively high in decolorization efficiency with good in both power conversion and COD removal efficiency and the final overall performance lies well within the range of those reported in the literature.

The power conversion efficiency and waste treatment efficiency (COD removal and decolorization efficiency) which was surveyed from many literature review was not effective due to the low surface roughness, low conductivity, low interaction between microbe and electrode, low electrocatalytic activity, and low cycle stability of anode in doubled chambered MFC. The presence of such challenges on previous work are addressed in this dissertation by modifying anode of MFC with simple, ecofriendly, and non-toxic biosynthesized α -MnO₂ based composites. Clearly, the α -MnO₂/PANI/PGE prepared in this work have remarkable biodegradation and power conversion enhancement. This material also features good decolorization and reusability.

Table 29. Comparison results obtained in the present work with the literature for the maximum power density, COD removal, and decolorization efficiency.

Substrate	Anode material	Dye	P _{max} (mW m ⁻²)	COD (%)	DE (%)	Reference
Acetate	MnO ₂ /NiO/CF	Methyl orange	628	58.29	92.5	[270]
Glucose	MnO ₂ /HNT/CC	-	767.3	93.1	-	[271]
Acetate	MgFe ₂ O ₄ /SS	Congo red	295.936	89.419	92.053	[272]
Glucose	NiCo ₂ O ₄ /PANI/CC	Methyl orange	~2.64	78±2.8	90 ±1.4	[273]
Glucose	PE/CF	Methyl red	282.77±15.95	-	-	[269]
DW	Cu-doped FeO/CP	-	161.5	75	-	[274]
Acetate	Lignin/CF	Acid Orange	120	58	81	[275]
Acetate	MnO ₂ /PPy-coated SS	-	440	100	-	[233]
SWW	MnO ₂ /PPy/ MWCNT/CC	-	1125.4	92	-	[276]
Glucose	α -MnO ₂ /PANI/PGE	Methyl red	820.02±7.86	88.19	95.57±2.26	This study

DW = Dairy wastewater, SWW = Sewage waste water, DE = Decolorization efficiency; HNTs = Halloysite nanotubes, PE = Polyethylenimine, CP = Carbon papers CF = Carbon felt; CC= Carbon cloth; SS = Stainless steel; MWCNT = Multi-walled carbon nanotube

4.3. Reusability of α -MnO₂/PANI/PGE and α -MnO₂/NiO/PANI/PGE

Reusability is an important performance index for assessing the practical applicability of α -MnO₂/PANI/PGE and α -MnO₂/NiO/PANI/PGE. In order to study the reusability of these synthesized electrodes, recycling experiments were conducted for three cycles. In order to

reuse the electrode repeatedly, both α -MnO₂/PANI/PGE and α -MnO₂/NiO/PANI/PGE were separated from the anode bioreactor, they were treated with 1 M NaOH for 10 minutes at 100 °C and then was ultrasonicated for 20 minutes. After wards, they were treated with 0.2 M PBS and dried for 12 hours at 60 °C using oven drying. Then, the treated electrodes were used to degrade glucose at the initial concentration and temperature of 160 mg L⁻¹ and 28 °C, respectively, by injecting 1 mL of *E. coli* in anode chamber of MFC for each cycle. As shown in Figure 72, after three consecutive cycles, the COD removal efficiency of α -MnO₂/PANI/PGE still remained over 80% with not much significantly different. On the contrary, the COD removal of α -MnO₂/NiO/PANI/PGE drastically decreased after the first run compared with α -MnO₂/PANI/PGE; this was probably owing to more glucose residuals covering the active surface after regeneration and decreases biofilm conduction, resulting from the low interaction between biocatalyst and nanocatalyst. This is the reason for slow electron transfer rate and causes for low power output and low removal efficiency. These results suggest that α -MnO₂/PANI/PGE could be regarded as a high-performance and reusable synthesized anode for glucose biodegradation from aqueous solution.

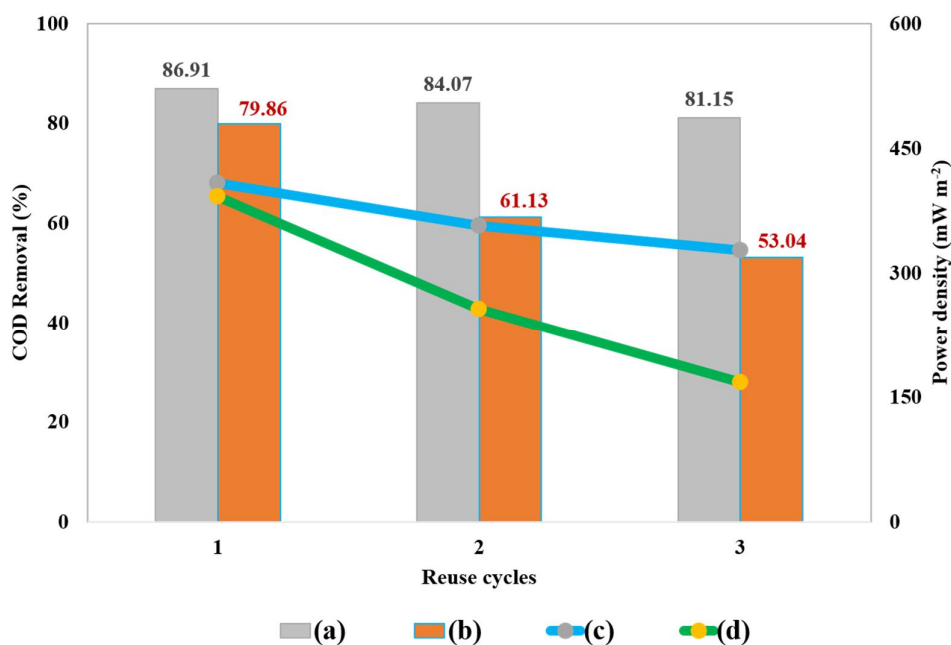


Figure 72. Reusability of the catalyst on glucose biodegradation for three cycles by α -MnO₂/PANI/PGE COD removal (a), α -MnO₂/NiO/PANI/PGE COD removal (b), α -MnO₂/PANI/PGE power density (c), and α -MnO₂/NiO/PANI/PGE power density (d). [Resistance 1000 Ω , time 180 minutes, glucose = 160 mg L⁻¹].

The findings of the present research suggested that the use of a cost-effective, environmental friendly, and reusable biosynthesized α -MnO₂ based PANI composite catalyst modified PGE should considerably increase the power production and treatment efficiency. The high power production, good COD removal with high coulombic efficiencies achieved in this research using such composite modified PGE suggested that there is potential to use this composites as an effective anode modifier for the use of MFC systems for future economical energy generation with a simultaneous solution for wastewater treatment that is discharged from various waste sources. According to these results, the biosynthesis of optimized non-noble based α -MnO₂ NPs compositing with conducting polymers strategy is a highly promising green method than conventional materials to reduce cost, time, and environmental hazard for the future scalable renewable energy conversion and toxic azo dye removal in MFC device system.

5. CONCLUSIONS

Biosynthesis of α -MnO₂ as well as its bimetallic Mn-Ni oxide based NPs were prepared through simple, ecofriendly, and non-toxic *V. amygdalina* plant leaf mediated approach. The basic experimental factors during ease of synthesis were optimized and analyzed with response surface methodology to increase the reliability and validation of synthesized materials and designed model. Accordingly, those optimized NMs were used as a starting material to prepare α -MnO₂/PANI and α -MnO₂/NiO/PANI nanocomposites.

The thermal, optical, structural, surface, and electrochemical characterizations of all catalysts were well reformed. Voltammetric and impedance analysis proved that α -MnO₂/PANI and α -MnO₂/NiO/PANI catalyst modified anode were found to have effective in electrocatalytic activities towards anodic oxidation reaction than non-composite modified one. It is extended that evidences from the interfacial properties proved by EIS between composite based electrode surface and PBS supporting electrolyte were promoted by the increased electrical conductivity of α -MnO₂/PANI/PGE composite. The lower charge transfer resistance and high electrical conductivity observed by α -MnO₂/PANI/PGE composite facilitates heterogeneous electron transfer rate due to having best and effective electroactive area that helps to validate maximum MFC performance.

To differentiate the better nanocomposites from α -MnO₂/NiO, α -MnO₂/PANI, and α -MnO₂/NiO/PANI under the same protocol, glucose and *E. coli* based artificial wastewater was used as a test model. The maximum V_{OC} produced by α -MnO₂/PANI/PGE was highest with best in cycle performance with a maximum power density of $426.26 \pm 38.89 \text{ mW m}^{-2}$ which has higher value than α -MnO₂/NiO/PGE ($412.24 \pm 5.98 \text{ mW m}^{-2}$) but lower than that α -MnO₂/NiO/PANI/PGE ($506.96 \pm 7.31 \text{ mW m}^{-2}$). That is in α -MnO₂/NiO/PANI composite modified PGE showed great potential as anode material during polarization, but a decline in treatment efficiency under external resistors (100 k Ω -100 Ω) was observed over time as compared to α -MnO₂/PANI/PGE. In contrast coulombic efficiencies increased considerably from 10.30% CE to 26.09% CE over time while COD removal also increased from 81.92% to 88.19%. Finally, α -MnO₂/PANI/PGE was selected as a best electrode for further remediation applications in MR based double chamber MFC device system and $820.02 \pm 7.86 \text{ mW m}^{-2}$ superior power densities was produced with better decolorization efficiency of $95.57 \pm 2.26\%$.

6. FUTURE RESEARCH AND RECOMMENDATIONS

From an overall outlook, due to economic constraints, a full characterization of synthesized materials (optimizing various parameters in the binary and ternary composite system) and composite modified anode was not possible over the long term in this study. However, this is highly recommended in any future studies performed on effective bioelectrochemical system. A complete chemical characterization of prepared nanomaterials and full component optimization in MFC setup would provide a holistic overview of experimental factor presence and would possibly identify those constraints which might minimize the power production and treatment efficiencies. Within this frame, throughout this study some parameters that enhance electrical energy conversion and biodegradation in terms of power density, COD removal, decolorization and coulombic efficiencies were continuously monitored by controlling pH, temperature, time, and external resistance. But some secondary parameters, such as substrate type, cathode electrode, concentration of catholyte, nature of salt bridge, and biocatalyst type were taken as constant. It is therefore suggested that future applications in MFC should consider to optimize those secondary parameters for efficient power conversion and biodegradation efficiency in MFC. Possibly those of the greatest inefficiencies in the current composite modified anode with in MFC were Ohmic and concentration losses. Their effect minimizes the performance of modified electrode. Preparing highly efficient catalyst and a complete removal of dissolved oxygen could help to enhance performances on MFC in future experiments. Therefore, it is highly recommended that further operational parameter optimization would be beneficial for future electrical energy production and waste treatment.

7. LIST OF PUBLICATIONS

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9. APPENDIXES

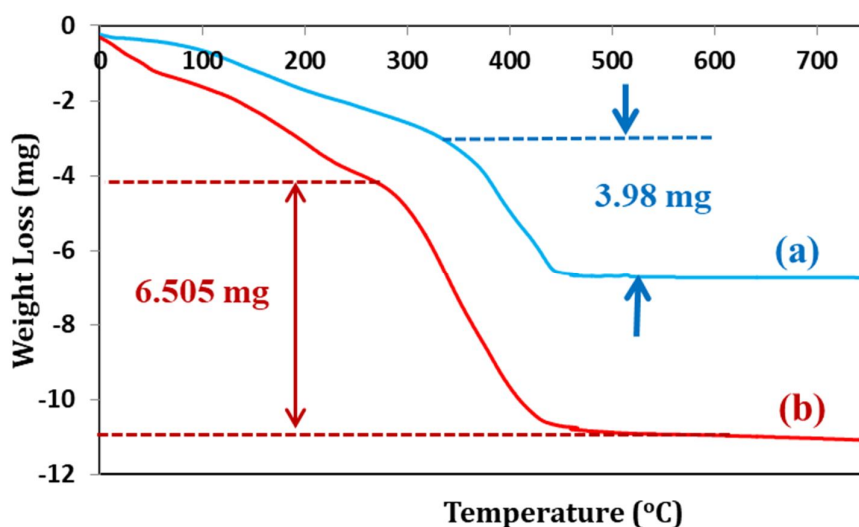


Figure A1. TGA of optimized uncalcined (a) MnO₂ and (b) MnO₂/NiO samples.

From the scan a wavelength at 526 nm is chosen as $\lambda_{\max}$. So, at this wavelength absorbance as a function of standard concentrations were plotted to determine the calibration curve and absorbance = 2.45155 x concentration (mM) + 0.01017.

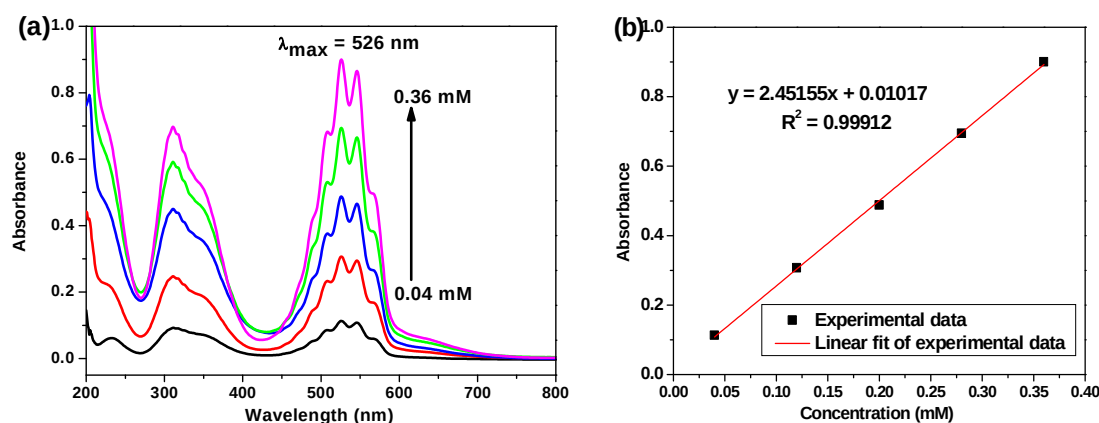


Figure A2. (a) Absorbance *versus* wavelength for 0.04, 0.12, 0.20, 0.28, and 0.36 mM standard solutions and (b) The extended calibration curve for laboratory prepared standard permanganate solutions at $\lambda_{\max} = 526$ nm.

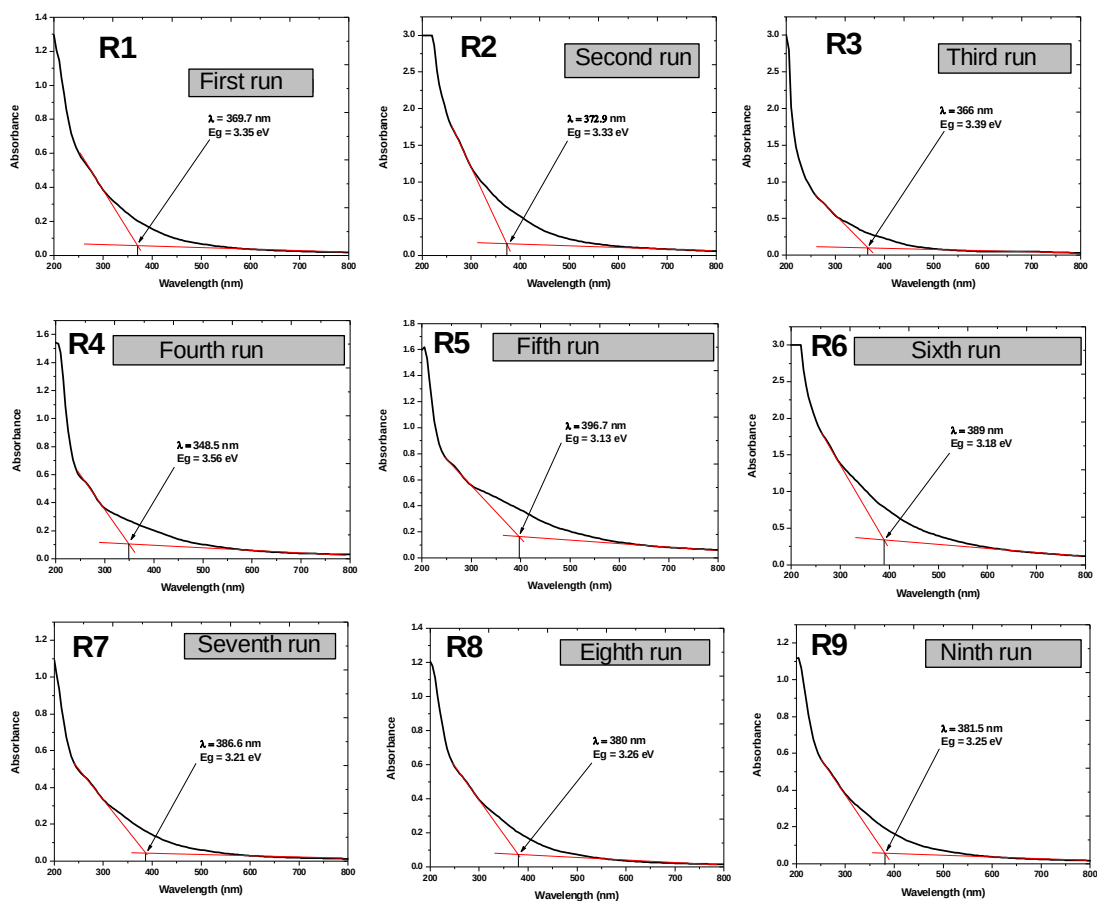


Figure A3. UV-Vis spectrum of 9 experimental runs for biosynthesized α -MnO₂/NiO nanocatalyst (R stands for experimental run).

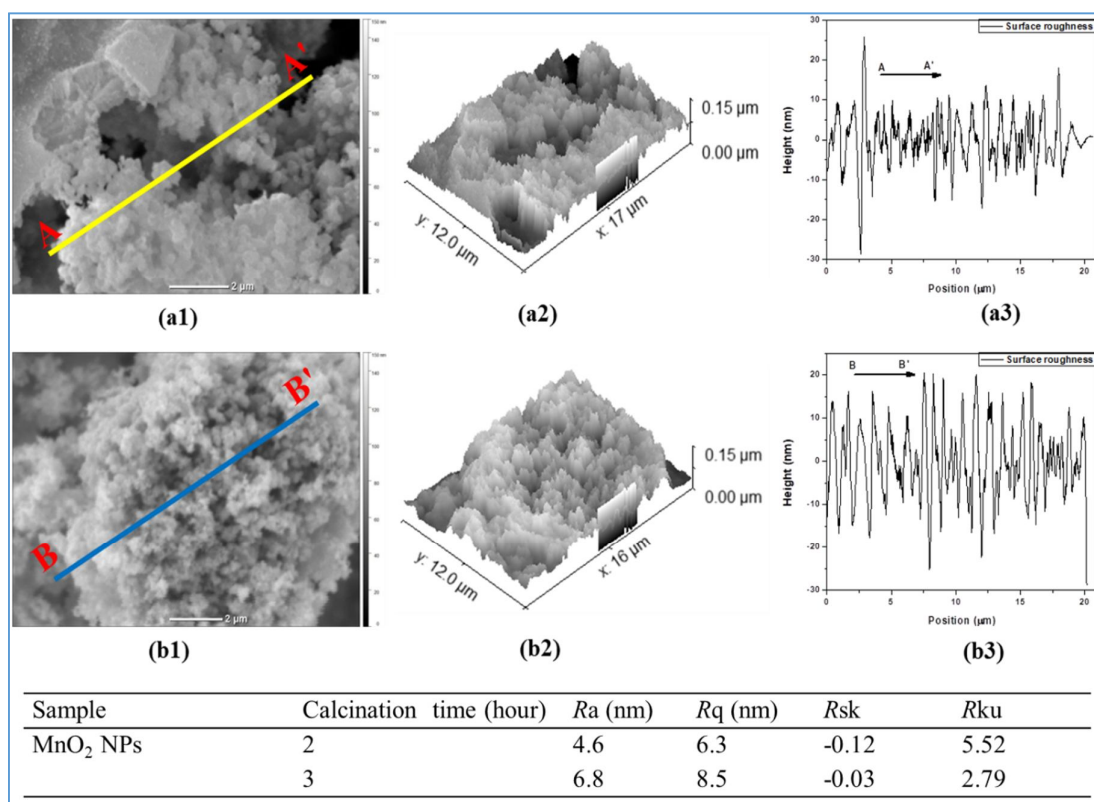


Figure A4. 2-D, 3-D SEM images of two α -MnO₂ NPs and diagonal section analysis of 2-D images calcined at 450 °C: (a1), (a2), (a3) after 2 hour calcination time and (b1), (b2), (b3) after 3 hour calcination time and roughness parameters.

Table A1. Summary of triplicate final concentration (COD_f) and their COD removal efficiency values for different anodic electrode during V_{OC} measurements under optimum conditions.

Anode electrode	Experiment 1 (COD _f mg L ⁻¹)	Experiment 2 (COD _f mg L ⁻¹)	Experiment 3 (COD _f mg L ⁻¹)	Mean ± Sd (mg L ⁻¹)	COD(%)
PGE	59.29	60.01	57.84	59.05 ± 1.11	64.43
α -MnO ₂ /PGE	34.77	35.25	34.06	34.69 ± 0.60	79.10
PANI/PGE	33.50	32.96	33.23	33.23 ± 0.27	79.98
α -MnO ₂ /PANI/PGE	12.88	13.12	12.53	12.84 ± 0.29	92.27
α -MnO ₂ /NiO/PANI/PGE	41.05	38.39	36.74	38.73 ± 2.17	76.67

(Mean ± Sd) is calculated using OriginPro 8 software, Sd = Standard deviation, substrate = (glucose, COD₀, 166 mg L⁻¹)

Table A2. Summary of triplicate final concentration (COD_f) and their COD removal efficiency values for different anodic electrode during CCV measurements under optimum conditions.

Anode electrode	Experiment 1 (COD _f mg L ⁻¹)	Experiment 2 (COD _f mg L ⁻¹)	Experiment 3 (COD _f mg L ⁻¹)	Mean ± Sd (mg/L)	RE (COD (%))	CE (%)
PGE	50.92	51.20	62.39	54.84 ± 6.54	67.00	2.29
α-MnO ₂ /PGE	40.60	41.14	42.51	41.42 ± 0.98	75.08	10.09
PANI/PGE	39.32	32.83	33.08	35.08 ± 3.67	78.89	14.54
α-MnO ₂ /PANI/PGE	19.97	20.31	18.57	19.62 ± 0.92	88.19	26.09
α-MnO ₂ /NiO/PANI/PGE	31.59	28.10	30.37	30.02 ± 1.77	81.92	10.30

(Mean ± Sd) is calculated using OriginPro 8 software, Sd = Standard deviation, substrate = (glucose, COD₀, 166 mg/L)

Note: both COD removal and CE efficiency were calculated from the mean values of COD_f

Table A3. Summary of kinetics, decolorization efficiency (DE) (%), removal amount (mg L⁻¹), and removal efficiencies (RE) (%).

Time (hour)	C ₀	A ₀	A _t	A ₀ -A _t	A ₀ -A _t /A ₀	DE	C _t	C ₀ /C _t	Ln (C ₀ /C _t)	C ₀ -C _t	C ₀ -C _t /C ₀	RE
0	50	0.362	0.362	0	0	0	50	1	0	0	0	0
	100	0.403	0.403	0	0	0	100	1	0	0	0	0
	150	0.463	0.463	0	0	0	150	1	0	0	0	0
	200	0.539	0.539	0	0	0	200	1	0	0	0	0
2	50	0.362	0.158	0.204	0.5635	56.35	21.37	2.339	0.85	28.63	0.5726	57.26
	100	0.403	0.157	0.246	0.6112	61.12	41.07	2.435	0.89	58.93	0.5893	58.93
	150	0.463	0.279	0.184	0.3971	39.71	93.75	1.599	0.47	56.25	0.3749	37.49
	200	0.539	0.376	0.163	0.3023	30.23	157.33	1.271	0.24	42.67	0.2134	21.34
4	50	0.362	0.099	0.263	0.7243	72.43	11.61	4.306	1.46	38.39	0.7678	76.78
	100	0.403	0.087	0.316	0.7842	78.42	19.79	5.053	1.62	80.21	0.8021	80.21
	150	0.463	0.205	0.258	0.5567	55.67	78.31	1.916	0.65	71.69	0.4779	47.79
	200	0.539	0.292	0.247	0.4581	45.81	127.53	1.568	0.45	72.47	0.3624	36.24
6	50	0.362	0.055	0.307	0.8479	84.79	6.01	8.331	2.12	43.99	0.8799	87.99
	100	0.403	0.039	0.364	0.9011	90.11	9.63	10.381	2.34	90.37	0.9037	90.37
	150	0.463	0.138	0.325	0.7008	70.08	34.15	4.393	1.48	115.85	0.7724	77.24
	200	0.539	0.214	0.325	0.6025	60.25	83.79	2.387	0.87	116.21	0.5811	58.11