CHAPTER I

INTRODUCTION

I.1 Background

As the world moves toward a critical inflection point in energy transition, fossil fuel consumption is projected to peak within this decade before declining, driven by resource depletion and increased sustainability efforts (Toru & Musa, 2023). This trajectory has sparked significant global interest in alternative energy systems that are not only renewable but also cost-effective and environmentally sustainable. Among these innovations, microbial fuel cells (MFCs) have gained attention for their unique ability to generate electricity through microbial metabolism. MFCs operate by oxidizing carbon-based substrates and transferring electrons to an external circuit via an anode (Pant *et al*., 2010; Logan *et al*., 2019). A particularly promising variant, the yeast-based microbial fuel cell (YMFC), utilizes *Saccharomyces cerevisiae* as a biocatalyst due to its safety, accessibility, and metabolic versatility (Boas *et al*., 2022; Verma & Mishra, 2021).

Saccharomyces cerevisiae possesses a facultative anaerobic nature, enabling it to thrive in both oxygen-rich and oxygen-limited environments, a feature that supports its robust performance in MFC systems (Gunawardena *et al*., 2008). Beyond its adaptability, yeast can facilitate extracellular electron transfer (EET) either directly or via redox mediators, making it suitable for sustainable bioelectricity generation (Duarte & Kwon, 2020; Christwardana *et al*., 2018a). However, despite its advantages, the electron transfer efficiency in YMFCs remains low due to the limited conductive interface between microbial membranes and

electrodes (Włodarczyk & Włodarczyk, 2023). Enhancing this transfer is thus central to improving MFC performance.

Various strategies have been proposed to address this bottleneck, ranging from optimizing biocatalysts and operating conditions to modifying electrode surfaces (Kumar *et al*., 2019; Santoro *et al*., 2017). While most attention has been placed on the cathodic catalyst—such as the substitution of platinum with iron-nitrogen-carbon (Fe–N–C) composites (Tang *et al*., 2016; Burkitt *et al*., 2015)—significantly less emphasis has been given to the anode, despite its vital role in mediating microbe–electrode interactions (Mustakeem, 2015; Li *et al*., 2011).

Carbon nanotubes (CNTs) offer a highly promising anodic substrate due to their high electrical conductivity, mechanical resilience, large surface area, and favorable biocompatibility (Zhao *et al*., 2016; Wang *et al*., 2018). These one-dimensional nanostructures provide an ideal scaffold for biofilm formation and efficient charge transport, making them excellent candidates for use in next-generation MFCs (Zhou *et al*., 2017). However, pristine CNTs suffer from limited functional groups, which restrict their microbial adhesion capabilities. Therefore, surface modification of CNTs becomes a necessary step to unlock their full potential.

One effective strategy to enhance the surface chemistry of CNTs is functionalization with polyethyleneimine (PEI)—a cationic, amine-rich polymer known to promote microbial adhesion and electrochemical activity (Christwardana *et al*., 2017; Gupta *et al*., 2021). PEI forms a positively charged layer over CNTs, improving both hydrophilicity and compatibility with negatively charged microbial

membranes (Yu *et al*., 2020). In addition to its adhesive properties, PEI provides multiple coordination sites for metal ions, laying the foundation for nanoparticle growth and electrocatalytic enhancement (Yang *et al*., 2019).

To further augment the conductivity and surface reactivity of PEI-CNT electrodes, gold nanoparticles (AuNPs) are often introduced due to their superior electrical, catalytic, and biocompatible properties (Chen *et al*., 2016; Fan *et al*., 2010). AuNPs exhibit excellent electron mobility, tunable size and morphology, and strong affinity for thiol and amine groups, allowing them to anchor well onto PEI-functionalized surfaces (Gupta *et al*., 2016; Boca *et al*., 2011). Their ability to enhance local charge density and facilitate rapid EET makes them ideal nanostructures for MFC applications (Alatraktchi *et al*., 2014). Beyond their role in electrochemical devices, AuNPs have garnered attention in diverse fields such as biosensing, drug delivery, photothermal therapy, and medical diagnostics, owing to their surface plasmon resonance and high surface-to-volume ratio (Thies *et al*., 2018; He *et al*., 2013). Their biological inertness and resistance to oxidation further amplify their utility across disciplines, reinforcing their strategic importance in sustainable nanotechnology platforms.

Interestingly, PEI is also known to possess weak reducing properties under controlled conditions (Yang *et al*., 2019). A study by Kosmella and Koetz (2006) demonstrated that polyethyleneimine (PEI) can function both as a reducing and stabilizing agent in the formation of gold nanoparticles (AuNPs). In their research, PEI was employed to reduce Au^{3+} ions to elemental gold (Au^{0}) without the need for any additional reducing agents, yielding nanoparticles with an average diameter of

approximately 7.1 nm. Furthermore, PEI played a critical role in stabilizing the synthesized particles by preventing aggregation and allowing redispersion after solvent evaporation. Additionally, PEI functioned as a capping agent by binding to the nanoparticle surface through its amine groups, forming a protective layer that regulated particle size and surface charge (Kosmella & Koetz, 2006). However, this reaction is often incomplete or slow, particularly in the absence of a co-reducing agent or external energy input. Moreover, PEI's lack of strong electron-donating groups limits its efficiency in achieving complete nucleation and dispersion of AuNPs on CNT surfaces, leading to potential aggregation or irregular particle growth (Zhou *et al*., 2017).

To overcome these limitations, lime juice (*Citrus aurantiifolia*) emerges as a sustainable and effective bioreductor, rich in L-ascorbic acid and natural phytochemicals such as phenolics, flavonoids, terpenoids, and alkaloids, which are capable of reducing metal salts to zero-valent nanoparticles (Ahmed *et al*., 2016; Singh *et al*., 2022). L-ascorbic acid (vitamin C) donates electrons to Au^{3+}, facilitating its reduction to Au^{+} and subsequently Au^{0}, while simultaneously capping the nanoparticles to prevent agglomeration (Khan *et al*., 2020). This green synthesis strategy not only eliminates the need for hazardous chemicals but also enables nanoparticle growth under ambient conditions, making it both environmentally benign and cost-effective.

PEI and *Citrus aurantiifolia* exhibit a complementary dual-reduction mechanism that promotes more stable and controlled nanoparticle formation. PEI, while capable of donating electrons through its amine groups, reduces Au^{3+} to Au^{0}

at a slower rate; the presence of L-ascorbic acid and phytochemicals in lime juice—such as phenolics, flavonoids, alkaloids, and terpenoids—accelerates the reduction process efficiently (Khan *et al*., 2020; Singh *et al*., 2022). This multistep mechanism enhances the interaction between gold ions and the PEI-functionalized CNT surface, ensuring that the reduction of Au^{3+} occurs in a spatially confined manner on the CNT/PEI interface rather than freely in solution (Ahmed *et al*., 2016). Consequently, the resulting Au^{0} nanoparticles are more efficiently anchored, better distributed, and more stable—an essential feature for anode applications in MFCs, where particle uniformity, electrochemical activity, and long-term stability are critical for enhancing extracellular electron transfer and promoting robust biofilm formation (Li *et al*., 2021).

Therefore, this research proposes a novel multistep reduction mechanism for synthesizing AuNPs onto CNT/PEI frameworks, combining the slow, stabilizing reduction ability of PEI with the rapid, phytochemical-rich reducing power of lime juice (*Citrus aurantiifolia*). By exploring synthesis durations of 5, 30, and 60 minutes, the study aims to investigate how nanoparticle morphology, distribution, and growth kinetics affect electrochemical characteristics and overall MFC performance. The work utilizes both half-cell and full-cell setups to examine microbial–electrode interactions, quantify voltage and power outputs, and evaluate biofilm formation on different anode surfaces. Ultimately, this study seeks to address existing gaps in anode optimization for YMFCs by integrating green-synthesized nanostructured electrodes, multistage reduction chemistry, and

biocatalytic engineering into a unified and sustainable platform for advanced bioenergy innovation.

I.2 Research Objectives

1. To analyze the structural characteristics of CNT, CNT-PEI, and CNT-PEI-AuNPs composites using appropriate characterization techniques.
2. To investigate the influence of synthesis duration (5, 30, and 60 minutes) on the electrochemical properties of the CNT-PEI-AuNPs-modified anode.
3. To evaluate the impact of CNT-PEI-AuNPs synthesized at different durations (5, 30, and 60 minutes) on the performance of a yeast-based microbial fuel cell (MFC), particularly in terms of power output and electron transfer efficiency.