EVALUATION OF POROUS SILICON FOR SUSTAINABLE MICROBIAL FUEL CELLS USING LACTIC ACID AND ELECTROACTIVE BACTERIA

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Abstract

Through a focus on its structural integrity, biocompatibility, and resistance to biofouling, this study evaluated the feasibility of using porous silicon (PSi) as a sustainable anode material for microbial fuel cells (MFCs). This work investigated the interactions between PSi and three bacterial strains: Lactobacillus acidophilus, Streptococcus thermophilus, and Bacillus subtilis, using a range of pore morphologies, including microporous, macroporous, nanoporous, and hierarchical (GPC/HPC) architectures. Scanning electron microscopy (SEM) was used to assess biofilm formation and structural integrity following exposure for 24 hours, 14 days, and 1 month.

The results demonstrated that PSi exhibits no symptoms of degradation or clogging while maintaining mechanical stability and pore integrity, hence supporting robust bacterial colonisation. Bacillus subtilis investigations further proved PSi's non-toxicity and ability to sustain biofilms, while the acidic pH of bacterial suspensions demonstrated successful microbial activity. These findings showed that PSi is a feasible and cost-effective alternative to conventional carbon-based electrodes, offering better scalability and performance for MFC applications. By addressing significant electrode design concerns, such as long-term durability and efficient electron transmission, this thesis provides a comprehensive analysis of the suitability of PSi to bioelectrochemical systems and advances the creation of sustainable energy solutions.

Keywords*: Microbial fuel cell, porous silicon, biocompatibility, bioelectrochemical systems, renewable energy, biofilm, proton exchange membrane.*

INTRODUCTION

Biomass to Fuel Cells: Biomass has been in existence for a very long time, dating back to the eras before civilization and technology, and they are byproducts of plant and animal waste. Biomass was previously only used for heating and cooking, but since the 19th century, new applications for biomass materials have emerged. Bioenergy, which makes up 98 % of renewable energy, is the kind of energy produced from biomass or biofuels that contributes to a reduction in

greenhouse gas emissions, whi`ch have fewer effects on climate change and global warming because they replace fossil fuels that can harm the environment [1].

Energy systems, including fire, wind, water, wood, coal, oil, and gas, have evolved from ancient times demonstrates the human demand for energy and the constant search for new ways to address energy-related issues.

Researchers are currently investigating the novel field of Microbial Cell Fuels (MFC) to address global energy issues and meet both human and environmental energy demands while reducing global warming. The power density that an MFC can normally produce ranges from 1 to 2000 mW m^{-2}, making waste-to-energy a significant strategy that several nations, including Sweden, are currently investigating [3].

Microbial cell fuels have several limitations that discourage energy consumers and researchers from exploring this method of electricity generation, despite the fact that they can be advantageous to both the environment and people. These restrictions are usually as follows:

- Current instability and high internal resistance
- Cost of MFC material fabrication and electricity production.
- Membrane fouling and low rate of microbial growth limit the application of MFCs [4].

to the present because they are crucial for sustaining society and promoting economic prosperity. Approximately 34 percent of greenhouse gas emissions generated by humans are linked to the energy sector, which is dominated by fossil fuels [2]. These energy systems

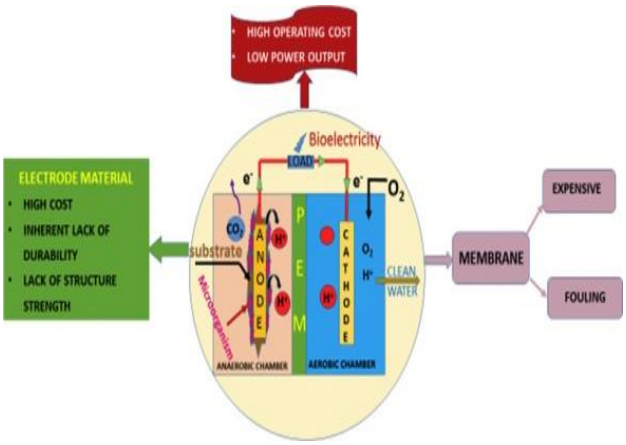


Figure 1 – The basic problems associated with MFCs [4]

As more research is conducted to address potential issues that may occur when employing MFC to generate power and decontaminate wastewater, there are also promising techniques that could mitigate the challenges associated with MFC.

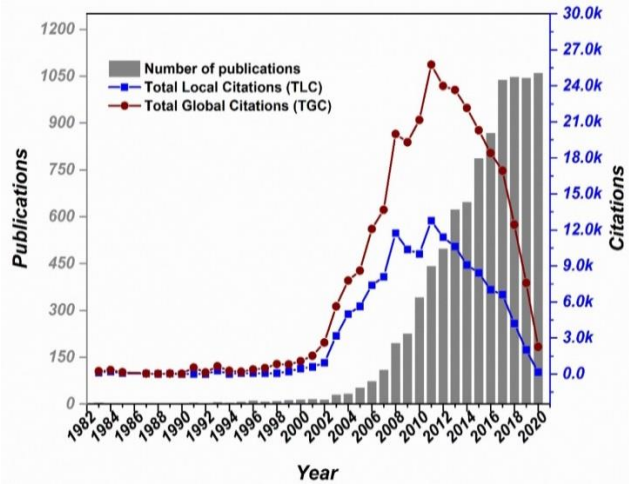


Figure 2 – Publication trend of MFCs over the years [5]

In recent years, researchers have been investigating MFCs to support green and

sustainable sources of energy, including 11,397 publications on Web of Science, 9,236 research articles and research review was about 1,267 [5]. Investigations on this source of energy are still a trending topic, as it is a viable source to reduce the dependence on fossil fuels and to protect the ecosystem.

Motivation: This dissertation is motivated by the use of renewable energy sources. The use of waste products that pollute the environment to generate electricity and decontaminate polluting materials is of particular interest. Fuel cells are ecofriendly and developing fields that can contribute to the energy sector.

Microbial fuel cells utilize gram-positive microorganisms such as *Lactobacillus acidophilus*, *Streptococcus thermophilus*, and *Bacillus subtilis* to generate electricity in microbial fuel cells under aerobic/anaerobic conditions and in water. However, their performance is limited by poor biofilm growth and electrode degradation. Porous silicon, with its high surface area and tunable morphology, offers a potential breakthrough if it is biocompatible and stable.

Objectives: The objectives of this study were as follows.

- Fabrication and characterization of porous silicon samples with various morphologies (macroporous, nanoporous, and GPC/HPC)
- The biocompatibility of PSi with *Lactobacillus acidophilus* and *Streptococcus thermophilus* (lactic acid bacteria) was assessed.
- To evaluate the compatibility and adhesion of *Bacillus subtilis* (electroactive bacterium) on PSi surfaces.
- To analyze the structural stability and pore preservation over 24 h, 14 d, and 1 month via SEM.
- To determine PSi's resistance of PSi to biofouling and its potential as a scalable anode material for MFC.

Novelty: This study investigated the use of both lactic acid and electroactive bacterial strains to test porous silicon as an MFC anode material while comparing multiple pore morphologies (including GPC/HPC) for long-term structural integrity in biological environments and to demonstrate no degradation or biofouling in PSi over extended exposure to bacterial cultures, supporting real-world MFC applications.

1 Literature Review

The history and various manufacturing processes for different membranes and biodegradable materials will be covered in this study, which will go deeper into the topic of microbial cell fuels. An interesting aspect of microbial cell fuels is that they allow the use of various techniques to achieve the same goal of producing electricity within a cell. The operating principle of MFCs is based on the most widely used membrane that has been investigated in multiple studies. This work will compare it to other membranes that are used less frequently and highlight the potential advantages of this membrane and other components needed to make an MFC function properly.

1.1 Basic Components of a Microbial Cell Fuel

The existence of animal electricity was established when an Italian scientist and physician

discovered that frog tissues possessed intrinsic potential for electricity [6]. In 1911, Michael C. Potter created the first microbial fuel cell (MFC) and showed that current could flow between two electrodes in a sterile medium for bacterial growth [7]. In 1977, a study [8] proposed the present MFC design approach. However, at the time, little was understood about how MFCs fecundated. The practical use of MFCs for the generation of electricity in 1999 was considered an important milestone in the advancement of technology [9].

The anode and cathode are two half-cells that constitute the MFC. A semi-permeable membrane material, often a proton exchange membrane (PEM), allows protons to travel from the anode to the cathode and separates the two half cells. The anodic chamber of the MFC is maintained in an atmosphere without oxygen, where microorganisms contribute to the production of protons and electrons. Carbon dioxide is produced as a byproduct of oxidation in the cathode chamber, as the electrons are transferred to the cathode after being absorbed by the anode. An external circuit was used to transfer the electrons [10]. An oxidizing substance, such as oxygen from free air, often makes up the cathode, which completes the reaction and closes the circuit. When the circuit is closed, often using a suitable load resistor, electrons can flow, transfer charge, and release the energy generated in the MFC [11].

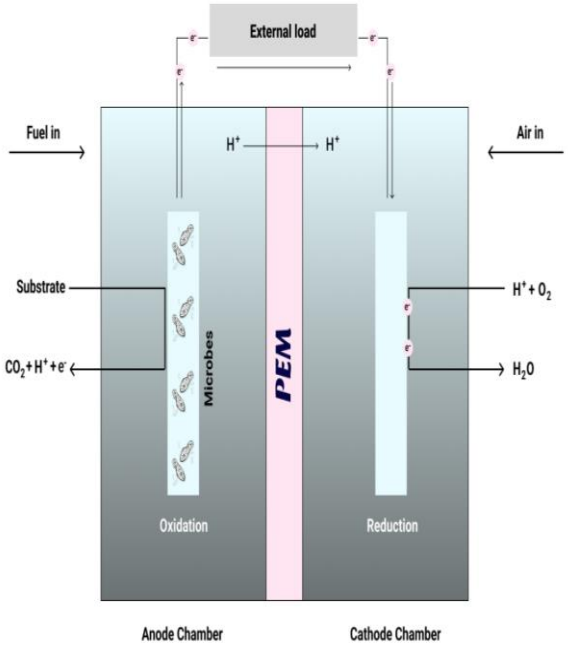


Figure 3 – An illustration showing the basic components of a MFC [10]

The efficiency of MFC depends on their construction. Factors such as substrate supply, temperature, biofilm formation, electrodes, and separator used in MFCs should be given close attention. Usually, the most important parameters can be considered as the electrodes (anode and cathode) and PEM [10].

1.1.2 Microorganisms

Bacterial culture is the basis for the use of microorganisms in MFC technology. Axenic cultures that are free of any other contaminating organisms and contain only one species or type of organism can be utilized in MFC [12].

Commonly used axenic cultures include *Shewanella putrefaciens*, *Clostridium beijerinckii*, *and Geobacter sulfurreducens*. Several mixed bacterial cultures have also been utilized, such as *Proteobacteria*, *Desulfuromonas*, *Clostridium butricum*, *and Bacteroides*, and

nitrogen-fixing bacteria, such as *Azospirillum* and *Azoarcus* [12].

Among Gram-negative microorganisms commonly used in MFCs are *Shewanella oneidensis*, *Bacillus violaceus*, *Escherichia coli*, *Geobacter metallireducens*, *Proteus vulgaris*, *Rhodoferax ferrireducens*, *Methylovorus mays*, *Shewanella affinis*, *Ochrobactrum sp.,* etc., [12].

In contrast, relatively few gram-positive bacteria have been reported in MFCs, including *Bacillus subtilis, Thermincola ferriacetica*, *Saccharomyces sp.*, *Clostridium beijerinckii*, *Bacillus cereus*, *Bacillus tequilensis*, *Corynebacterium sp.*, and *Paenibacillus lautus* [12].

Microorganisms, such as *Lactobacillus acidophilus* and *Streptococcus thermophilus,* can also be utilized in MFCs. Gram-positive bacteria are usually obtained from the fermentation of milk and the production of yoghurt and cheese. They can also be referred to as lactic acid bacteria (LAB), and are good examples of bacteria that encourage the development of biofilms [13].

1.2 Electrodes

For MFCs to function well in terms of bacterial adhesion, electron transfer, and electrochemical efficiency, the appropriate electrode material must be used. Many studies have been conducted to increase the production of electricity using various carbon-based materials, including composites made of carbon nanotubes, carbon paper, carbon felt, and carbon fiber. Power densities must be increased and material costs must be decreased to make MFCs practical and usable [14].

The selection criteria for materials used as the anode and cathode in MFCs are different; in general, both should have good electrical conductivity, be stable and durable, be porous and have a large surface area, and be inexpensive and easily accessible. In addition, the materials used for the anode must have biocompatible properties to improve biofilm growth and adhesion, which will help increase the efficiency and life span of MFCs in the long run [14].

1.2.1 The Anode

Choosing a suitable anode material for MFCs is important because it has a significant impact on the growth of biofilms and the transition of electrons between the microorganism and the electron acceptor [14]. Below is an overview of the different materials used in MFC, such as carbon rods, carbon cloth, carbon fiber, and stainless steel mesh.

Carbon nanotubes (CNTs): Carbon nanotubes (CNTs) can serve as excellent anode materials because of their large surface area, mechanical strength, stability, and conductivity. CNTs can increase the useful surface area and electron transport at the anode. Multi-walled CNT (MWCNT) are usually preferred over single-walled CNT (SWCNT) because of the extra hydroxyl groups that enhance electron transferability, biofilm development, and substrate diffusion and oxidation [15].

Conventional carbon-based materials: Because of their excellent conductivity, good chemical stability, advantageous biocompatibility, and relatively low cost, carbon-based materials such as carbon paper, carbon cloth, graphite plates, carbon meshes, activated carbon cloth, granular graphite, graphite felt, carbon felt, granular activated carbon, reticulated vitreous carbon (RVC), and carbon brushes are among the

materials most frequently employed for MFC anodes. To increase the efficiency of MFCs, carbon-based electrodes can be arranged in three different types: brush, packed, and plane structures. Each type has a different maximum power or current density [16].

Conductive polymer-based composite materials: By combining the efforts of active support, high electrical conductivity, and the number of catalytic active sites of the prepared rGO/PANI/Pt nanocomposite, scientists have demonstrated that using platinum (Pt) nanoparticles anchored over reduced graphene oxide (rGO) and rGO/conductive polyaniline (PANI) composites as anode catalysts in MFCs can result in a maximum MFC power density of 2059 mW/m2 with concrete life durability, making it a viable option as an anode material in MFCs [17].

Porous silicon: Special consideration is given to porous silicon because this material will be investigated in this study. In the 1950s, Uhlir (1956) and Turner (1958) initially discovered porous silicon (PSi) when electropolishing silicon wafers using an electrolyte containing HF. They discovered that the silicon did not dissolve evenly under the right applied current and solution composition circumstances; rather, tiny holes were created in porous silicon, as it is known today, because they contain pores [18]. In a later experiment, scientists found that charge carriers are drawn to the Si/HF contact by the electrical voltage between the cathode (platinum) and the anode (silicon). Injecting holes into the silicon surface causes a strong reaction with the Si-H bonds, which leads to the dissolution (etching) of Si. The porosity and uniform thickness of the Si wafer are the end results of the etching process [19]. The ability to adjust the pore size from a few nanometers to a few tens of micrometers by simply selecting the wafer doping level and etching conditions makes the electrochemical etching of silicon interesting [20]. The current definition (as well as IUPAC) distinguishes three categories of materials: microporous (less than 2 nm pore width), mesoporous (usually between 2 and 50 nm pore width), and macroporous (typically larger than 50 nm pore width). It is crucial to note that these types are determined by the pore size rather than the remaining structures [21]. With etching current densities of 5, 10, 15, 20, and 25 mA/cm^2, PSi demonstrated a nanostructure (mesoporous silicon) with decreasing pore diameter as the etching current density increased [22]. Additionally, in another experiment, researchers observed the development of pores with average diameters ranging from 36 to 48 nm. As the etching time increases, so does their average size; because of the HF attack, the pores become larger [19].

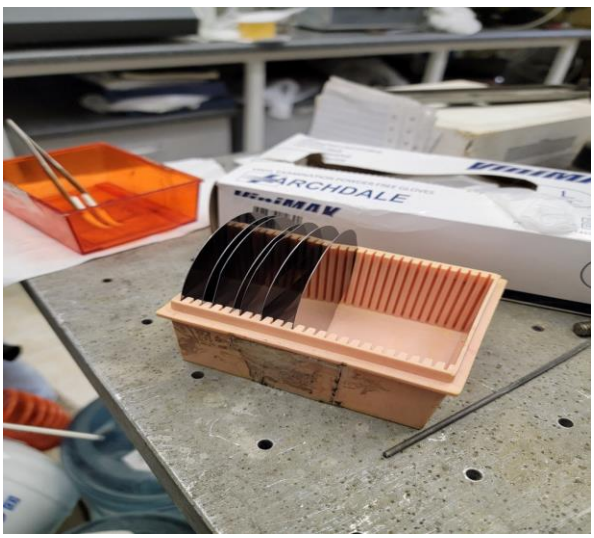


Figure 4 – Image showing a group of porous silicon samples

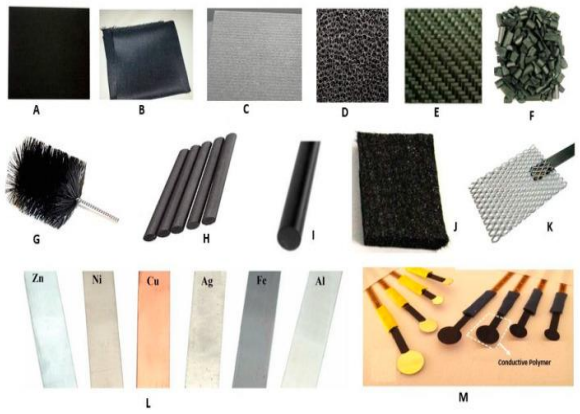


Figure 5 – Other commonly used electrodes for anode and cathode in MFCs [23]

A-carbon paper, B-carbon cloth, C-carbon fiber, D-reticulated vitrified carbon, E-carbon mesh, F-graphitic granular, G-carbon brushes, H-graphite rods, I-polycrystalline graphite J-carbon felt; K-platinum mesh; L-different metal electrode strips; M-conductive polymer-based strips.

1.2.2 The Cathode

Cathodes are important for assessing the performance of microbial fuel cells (MFCs), including power density and cost. In both the presence and absence of catalysts, MFCs employ a variety of cathode types, including air, aqueous air, and bio-cathodes [24]. An ideal cathode should have superior mechanical durability, excellent electrical conductivity, and excellent catalytic properties. Cathode materials can be based on carbon [25]. Pt catalysts are typically used as carbon-supported catalysts in fuel cells (FCs). Consequently, the surface area and durability increased. Several MFC systems with Pt/C cathode catalysts have achieved high power density. In addition, active carbon, graphene, and graphite materials have been employed as supports, together with catalysts made of non-precious metals. One-by-one MFCs can employ metal-based and carbon-based catalysts, which can be combined to create an appropriate cathode catalyst material [26].

Because the electrocatalyst in air-cathode-based MFCs is directly exposed to air, there is more oxygen available for the reaction. For protons or hydroxyl ions to move toward or away from the electrocatalyst, air cathodes need to be set up specifically to ensure quick oxygen transport to the active site while retaining contact with the electrolyte [24]. When creating air cathodes, carbon compounds and binders are utilized as conductive modifications for electron transfer or as current collectors [27]. An air cathode offers a good opportunity to increase power output through MFCs [28]. Conductive materials, such as platinum mesh, carbon felt, carbon fiber, and carbon cloth, are used to create electrodes for aqueous air cathodes. These electrodes are then attached to the catalyst layer, which is already present in aqueous regions with low oxygen contact [29]. Tetrafluoroethylene (poly) and perfluorosulfonic acid (Nafion) are the most commonly used binders. Zhang et al. [30] used poly (tetrafluoroethylene) as a binder to assess the performance of carbon cloth and activated carbon as cathode materials. The findings showed that when Pt was present as a catalyst, activated carbon had a higher power density than carbon cloth (1220 mW/m^2 in contrast to 1060 mW/m^2). Thus, activated carbon may be a suitable substitute for cathode material preparation [23].

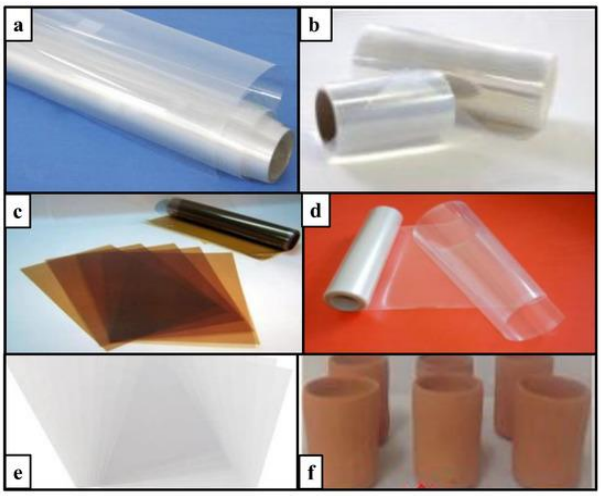
Aerobic biocathodes use microorganisms as biocatalysts for the cathode oxygen reduction reaction (ORR). To create high-performing aerobic biocathodes, biofilms were first grown in electrochemical half-cells positioned at -0.1 and +0.2 V. Biocathodes can outperform abiotic cathode catalysts in terms of performance because

they are less expensive, environmentally friendly, and protect against catalyst poisoning [31]. It requires aid for the bacteria to develop a biofilm for a biocathode to form. Stainless steel is frequently used for biocathode production [10]. Biocathode fabrication also uses carbon-based materials such as granular graphite, activated carbon, and graphite felt [32]. Increasing the surface area available for biofilm growth could increase the efficiency of biocathodes. The biocathode also improves performance by lowering the internal resistance [33]. Owing to their benefits, biocathodes are now receiving more attention, and research is being conducted to identify biocathodes that can increase MFCs performance at a lower cost.

1.3 Membrane

An essential feature of MFC design is that the membrane stops the mixture of reactions originating from the cathode and anode compartments. In addition, it restricts oxygen transported [34]. Additionally, the separator enhances the Coulombic efficiency (CE) of the MFC by preventing the flow of undesirable compounds into the reactor [35]. The parameters that determine if the internal load of the MFC increases or decreases depend on the type of membrane and its quality. A high internal load limits the practical use of MFCs and results in poor performance [36]. Although the selection of separators for MFCs has advanced significantly in recent years, researchers still face considerable difficulty in locating appropriate and reasonably priced membranes.

Nafion membranes are frequently used in MFCs because of their superior ionic conductivity. Commercial membranes, including cation and anion exchange membranes (CEMs; AEMs), bipolar membranes, microfiltration membranes, and nanoporous membranes have also been introduced to replace Nafion membranes. Other membrane types have also been studied, including clay cup and ceramic membranes [37].



a, Nafion 117; c, bipolar membrane; c, AEM; d, PEM; e, CEM; f, clay cup membrane.

Figure 6 - Common membranes used in MFCs [37]

1.4 Working mechanism of MFCs

MFCs are an example of a bioelectrochemical process that uses electrons from bacterially catalyzed metabolic reactions to generate electricity. The anodic and cathodic chambers of a regular MFC are divided by a proton exchange membrane [38]. The anode chamber, where microbes metabolize organic substrates (such as acetate) to liberate protons and electrons, is usually anaerobic.

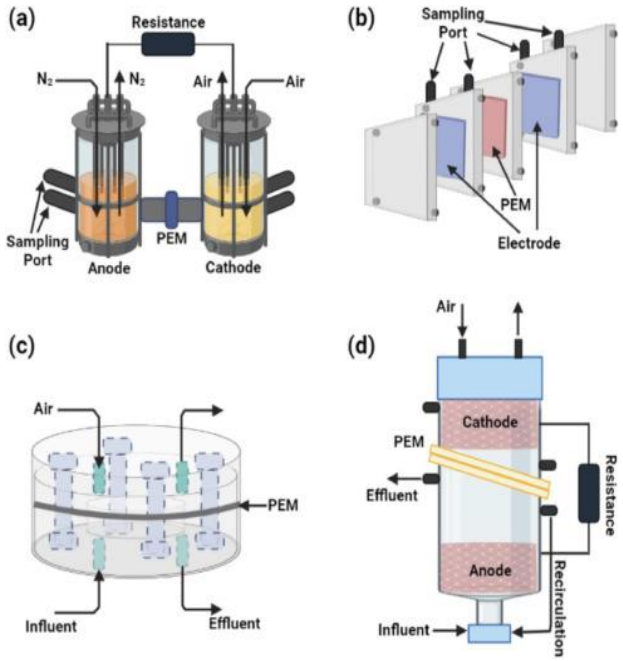
The anode receives the electrons released by the microorganisms and sends them to the cathode via an external circuit. A proton is transported across the membrane to the cathode for every electron to pass [39]. Oxygen is frequently used as an electron acceptor in MFCs in the cathode compartment. The transfer of protons and electrons generated in the cathode chamber results

in an electric current that reduces oxygen to water [31].

1.5 Types of MFC based on chambers

MFCs are generally classified according to their number of chambers, with each type having unique benefits and uses. The images that appear below show a simplified illustration of the most widely used MFCs along with the features and variations that accompany them. Because of their small size, affordability, and ease of scalability, single-chambered MFCs are preferred and can be used in places that are far away. Owing to the high rate of bioelectricity generation in dual-chambered MFCs, they are frequently used for pilot-scale bioelectricity generation.

Recent developments in mini-MFCs and up-flow MFCs have generated highly efficient power-density output designs. Despite their complexity, multi-chambered MFCs are ideal for in situ maintenance in real time. Naha et al. [40] further emphasize the usefulness of MFC in producing bioenergy as well as its other functions demonstrated by the efficient microbial strain that uses wastewater substrates as an energy source and transforms toxic substrates into less harmful forms. In the near future, these multi-chambered MFCs could be the answer to the impending energy issue if powerful PEM, mediators, and catholyte-anolyte combinations are optimized and used [41].



(a) Typical single-chambered MFC, (b) Tubular MFC with an outer cathode and inner anode comprising graphite granules, and (c) standard triple-chambered MFC.

Figure 7 - Illustrations of single and triple-chambered MFCs [42]

1.6 Electron transfer mechanism

The type of organic matter in the wastewater, the rate at which electrons move from bacteria to the anode, and the ability of the membrane to transfer hydrogen ions are some of the factors that affect the output power of an MFC [43]. MFC are bioelectrochemical systems that use exoelectrogenic bacteria grown on an anode to oxidize organic materials. An external circuit gathers the electrons at the anode and moves them to the cathode. Protons are created when organic matter is oxidized, and they move across a selective membrane from the anode to the cathode [44]. The ability to reproduce this has been observed in two significant bacterial genera, *Shewanella* and *Geobacter*. There are three methods by which electrons are transported extracellularly to the electrodes.

1. Electron transfer through mediators: A small percentage of bacterial species, including *Shewanella* and *Pseudomonas*, release chemical species termed shuttle molecules, including flavins, which move electrons from the bacterial outer membrane to electrodes [45].

2. Direct electron transfer: In the direct electron transfer mechanism, when a bacterial cell membrane touches a cathode, electrons are transferred directly between the two [25/new]. The *Shewanella* and *Geobacter* species possess the ability to transfer electrons directly, where electrons are transported straight to the electrode surface. The direct transfer of electrons generated from NADH is facilitated by the outer membrane cytochrome (C-type) [46].

3. Electron transfer through nanowires: Conductive appendages are used by Shewanella and Geobacter species to transfer electrons outside the cell [47]. These conductive networks, known as nanowires (NWs), are cellular extensions that can reach a length of 20 µm. According to previous reports, these nanowires have far greater electronic conductivity than synthetic metallic nanostructures [48]. The fourth route of electron transfer was suggested by Schaetzle et al. by oxidizing catabolites released by bacteria [49].

1.7 Literature review summary and Challenges to overcome

From their theoretical start in the early 20th century to their current position as promising bioelectrochemical technologies for sustainable energy generation and wastewater treatment, the historical development and technological evolution of microbial fuel cells (MFCs) have been methodically examined in this extensive literature review. According to the review, electrode materials, both the anode and cathode, play a crucial role in determining MFC performance. Their electrical conductivity, surface properties, and biocompatibility have a direct impact on the power output and system efficiency.

Important conclusions from the literature review show that strong biofilm growth and effective extracellular electron transfer (EET) must be supported by anode materials simultaneously. Carbon-based materials are currently leading research because of their cost-performance ratio. Owing to the high cost of platinum catalysts and the kinetics of the oxygen reduction reaction (ORR), the cathode design continues to be a significant bottleneck. Electroactive microorganisms exhibit a wide range of electron transfer mechanisms, from direct contact through membrane-bound cytochromes to mediate transmission via soluble redox shuttles or conductive nanowires. As membrane/separator technologies advance, proton exchange membranes (PEMs) encounter difficulties in biofouling and pH gradient generation.

The following section describes our experimental methodology for assessing PSi's electrochemical performance and biocompatibility of PSi in MFC applications with an emphasis on:

- Evaluation of the structural stability of PSi in the presence of microbial colonization.
- Evaluation of biofilm development kinetics
- Evaluation of the electron transfer efficiency in comparison with traditional carbon electrodes.

- Testing for long-term endurance in operational settings

By methodically tackling these issues, this study seeks to position PSi as a promising next-generation electrode material that can get past a number of the drawbacks found in existing MFC technologies, possibly opening the door to more effective and financially viable bioelectrochemical systems for the production of renewable energy.

2 Experimental Techniques and Theory

Porous silicon (PSi) is a material produced by the dissolution of n-type or p-type silicon wafers in a hydrofluoric acid (HF) solution. PSi prepared in HF-based solutions is a structure with pores, micro- and nanometer-sized crystallites, columnar nanostructures, spikes, nanowires, nanotubes, and nanorods embedded in a silicon layer. The PSi structure depends on several factors, such as the material conductivity, etching current density, and solution concentration. To form PSi with pores, a solution of HF with alcohol as a solvent was used [19].

2.1 Study of the available samples before placing them in the bacterial environment

The images labeled a to i below show the samples under study, which were pre-washed in alcohol before placement in the bacterial environment. The samples consisted of uncontaminated porous silicon with different pore morphologies.

In subsequent pages, a list of tables describing the SEM analysis of the samples in contact with a bacterial medium will be provided, and I have stated the full meanings of the abbreviations that will be used below.

- KDB: Kremlin-Doped Boron (a designation for boron-doped p-type silicon wafers), where KDB 9, 10, 1000, etc., indicate resistivity values (e.g., KDB 9 = ~9 Ω·cm), and KDB 0.005 and 0.002 indicate very low resistivity (heavily doped).
- HF: Hydrofluoric Acid (used for silicon etching).
- DMF: Dimethylformamide (organic solvent in the etchants).
- STAC: Stearyltrimethylammonium Chloride
- GPC: Gradient Porosity Column (pores vary in size across depth).
- HPC: Hierarchical Porosity Column (multiple pore sizes coexist).
- j: Current density.

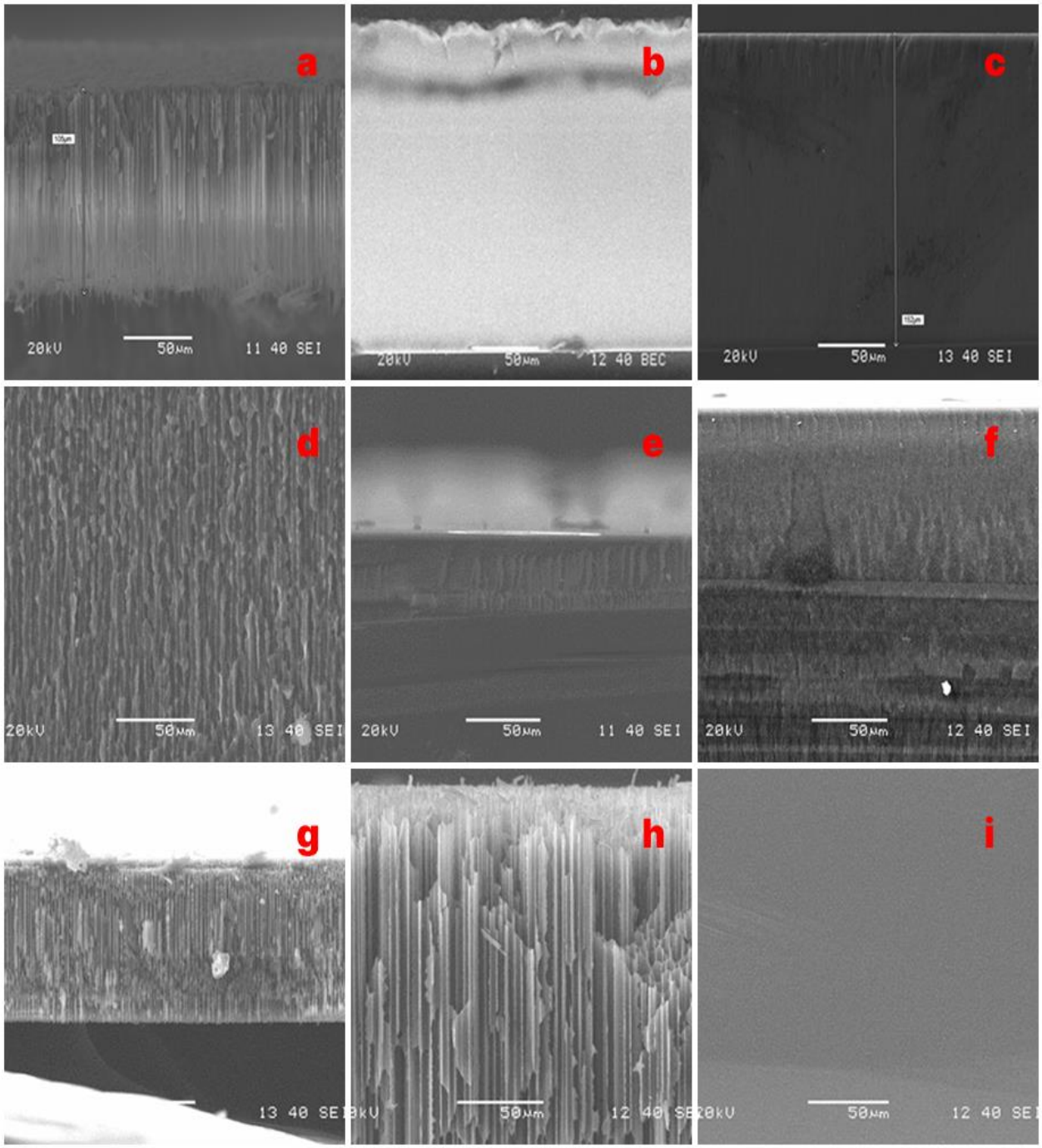


Figure 8 – SEM images of samples before placing them in the bacterial environment (see table 1)

Table 1 - Labels for SEM images of samples before placing them in the bacterial environment

Images	Description
a	KDB 9, HF:DMF=1:10, j = 15 $\frac{mA}{cm^2}$, t=300min, macroporous.
b	KDB 0.005, HF: C_2H_5OH=1:1, j = 100 $\frac{mA}{cm^2}$, t=50 min, nanoporous.
c	KDB 0.002, HF:C_2H_5OH=1:1+STAC, j = 80 $\frac{mA}{cm^2}$, t=60 min, nanoporous.
d	KDB 1000, HF:$CH_3CH(OH)CH_3$=6:1+STAC, j = 10 $\frac{mA}{cm^2}$, t=300 min, GPC-var.
e	KDB 10, HF: :DMF=1:6, j = 15 $\frac{mA}{cm^2}$, t=240 min, nanoporous.
f	KDB 10, HF:$CH_3CH(OH)CH_3$ = 6:1, j = 20 $\frac{mA}{cm^2}$, t=150 min, HPC-var.
g	KDB 10, HF:DMF=1:10, j = 15 $\frac{mA}{cm^2}$, t=240 min, macroporous.
h	KDB 10, HF:$CH_3CH(OH)CH_3$:H_2O, j = 20 $\frac{mA}{cm^2}$, t=240 min, microporous.
i	Monocrystalline silicon.

2.2 Preparation of reference bacterial medium

The model bacterial medium, due to the availability of the necessary components and the simplicity of the manufacturing technology, was made from the Acidophilin Bakzdrav starter culture, which contains strains of *Lactobacillus acidophilus* bacteria - a homofermentative anaerobic microorganism, that produces only lactic acid as an end product of fermentation [50], and *Streptococcus thermophilus* - a gram-positive bacterium and a fermentative facultative anaerobe of the viridans group that is non-motile and does not form endospores [51], and milk of the "Green Village" brand, due to the absence of antibiotics in it that prevent the development of a colony of bacteria as the pH of a fresh milk ranges from 6.4 to 6.9, the pH drops as the milk goes sour.

First, a reference suspension that did not contain any of the available samples was prepared. For this, 200 ml of milk and one packet of starter (5 g) were placed in a sterile container, followed by stirring to achieve homogeneity of the mixture. The prepared suspension was then placed in a drying cabinet to maintain a constant temperature, as shown in Figure 8. The suspension was maintained at 40 °C for 6 h to incubate the microorganisms [52].

Figure 9—Drying cabinet UT-4620

After this time, the reference bacterial medium was successfully obtained, as evidenced by the change in the consistency of the mixture (Figure 10) and pH meter readings (Figure 11). Because the preparation of the reference model medium was successful, it was decided to carry out the fermentation of milk in direct contact with the available samples of porous silicon.

Figure 10— The resulting reference bacterial medium, which has a viscous consistency

2.3 Preparation of a model bacterial medium in direct contact with existing porous silicon samples

To confirm the non-toxicity of porous silicon, a decision was made to conduct reference fermentation in contact with a non-toxic sample of monocrystalline silicon for further comparison of the obtained results with those obtained in contact with the samples under study.

Milk was fermented in direct contact with the available porous silicon samples using the method specified in Section 3.2. After the specified time, the containers were removed from the drying oven to analyze the fermentation results. The consistency of all suspensions containing porous silicon structures changed from liquid to more viscous [52] and turned out to be indistinguishable from the consistency of the suspension obtained in contact with monocrystalline silicon, which confirms the success of the incubation process and the biocompatibility of porous silicon. (Images a–h).

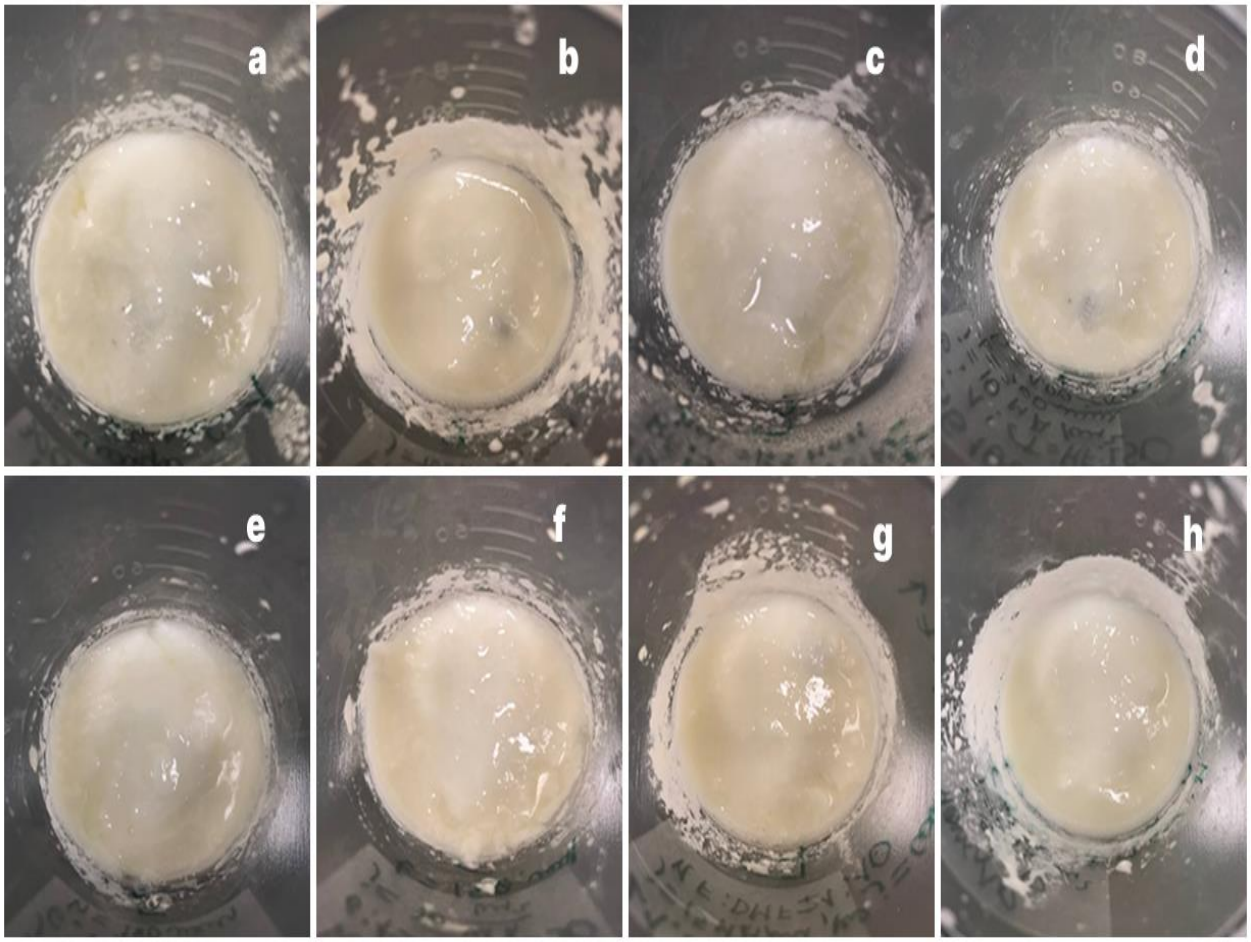


Figure 11 — porous silicon samples for fermented milk in bacterial medium (see table 2)

Table 2 - Labels of porous silicon samples for fermented milk in bacterial medium

Images	Description
a	KDB 9, HF:DMF=1:10, t=300 min, j = 15 $\frac{mA}{cm^2}$, macroporous.
b	KDB 0.005, HF: C_2H_5OH=1:1, j = 100 $\frac{mA}{cm^2}$, t=50 min, nanoporous.
c	KDB 0.002 HF: C_2H_5OH = 1:1 + STAC j = 80 $\frac{mA}{cm^2}$, t=60 min, nanoporous.
d	KDB 1000 HF:$CH_3CH(OH)CH_3$=6:1+STAC, j = 10 $\frac{mA}{cm^2}$, t=300 min, GPC—var.
e	KDB 10 HF:DMF=1:6, j = 15 $\frac{mA}{cm^2}$, t=240 min, nanoporous.
f	KDB 10, HF:$CH_3CH(OH)CH_3$ = 6:1, j = 20 $\frac{mA}{cm^2}$, t=150 min, HPC-var.
g	KDB 10 HF:DMF=1:10, j = 15 $\frac{mA}{cm^2}$, t=240 min, macroporous.
h	KDB 10, HF:$CH_3CH(OH)CH_3$:H_2O, j = 20 $\frac{mA}{cm^2}$, t=240 min, microporous.

A pH meter was used to confirm the presence of bacteria in the suspensions (Figure 10). The pH values of the suspensions are listed in Table 3. The acidity index values for various suspensions indicated that fermentation in contact with all studied samples was successful. As expected, *Lactobacillus acidophilus* and *Streptococcus thermophilus* bacteria contributed to the acidity of the suspensions [52] [58].

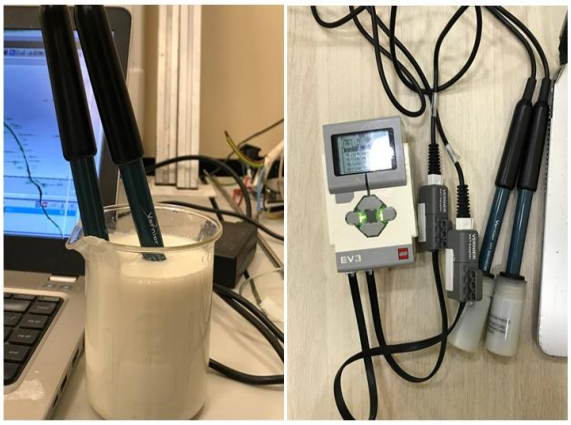


Figure 12—Measurement of pH of the obtained suspensions

Table 3 - pH values of the obtained suspensions

Suspension	pH value
KDB 9, HF:DMF=1:10, j = 15 $\frac{mA}{cm^2}$, t=300 min	3.06
KDB 0.005, HF: C_2H_5OH=1:1, j = 100 $\frac{mA}{cm^2}$, t=50 min	3.05
KDB 0.002 HF:C_2H_5OH=1:1+STAC, j = 80 $\frac{mA}{cm^2}$, t=60 min	3.01
KDB 1000 HF:$CH_3CH(OH)CH_3$=6:1+STAC, j = 10 $\frac{mA}{cm^2}$, t=300 min	3.09
KDB 10 HF:DMF=1:6, j = 15 $\frac{mA}{cm^2}$, t=240 min	3.29
KDB 10, HF:$CH_3CH(OH)CH_3$ = 6:1, j = 20 $\frac{mA}{cm^2}$, t=150 min,	3.13
KDB 10 HF:DMF=1:10, j = 15 $\frac{mA}{cm^2}$,t=240 min	3.06
KDB 10,HF:$CH_3CH(OH)CH_3$:H_2O, j = 20 $\frac{mA}{cm^2}$, t=240 min	3.08
Monocrystalline silicon	3.11
Reference bacterial medium	2.89
Milk "Green Village"	6.25

2.4 SEM analysis of samples after 24 hours of exposure to suspensions

On the first day after the preparation of suspensions in contact with the coatings, the samples were removed from the bacterial media for subsequent observation using SEM. After removing the samples, it was noted during the visual assessment that no decomposition or degradation of the structures occurred during fermentation.

The samples were thoroughly washed with alcohol to remove any dirt. After the sample preparation procedure, the samples were secured to the side surfaces of the cylindrical holders, with the end part facing the microscope, and placed on a stand in the chamber of the powder electron microscope.

When examining the samples using SEM, no degradation of the pores or structure was detected. The formation of bacterial films on the pore surfaces was recorded. The images below confirm the absence of degradation of porous silicon with different pore morphologies upon contact with a model bacterial environment during short-term exposure, and the ability of porous silicon to accommodate bacteria on its surface [53]. No samples unsuitable for use in fuel cells were identified, since all the studied samples demonstrated no tendency to degrade (images a to h) and the ability to attach bacteria. However, it was decided during the last iteration of the sample analysis to analyze the fresh chips to obtain a more detailed picture of the changes in the structure of the samples in the pore volume.

Images	Description
a	KDB 9, HF:DMF=1:10, t=300 min, j = 15 $\frac{mA}{cm^2}$, macroporous.
b	KDB 0.005, HF: C_2H_5OH=1:1, j = 100 $\frac{mA}{cm^2}$, t=50 min, nanoporous.
c	KDB 0.002 HF:C_2H_5OH=1:1+STAC, j = 80 $\frac{mA}{cm^2}$, t=60 min, nanoporous.
d	KDB 1000 HF:$CH_3CH(OH)CH_3$=6:1+STAC, j = 10 $\frac{mA}{cm^2}$, t=300 min, GPC—var.
e	KDB-10 HF:DMF=1:6, j = 15 $\frac{mA}{cm^2}$, t=240 min, nanoporous.
f	KDB 10, HF:$CH_3CH(OH)CH_3$ = 6:1, j = 20 $\frac{mA}{cm^2}$, t=150 min, HPC-var.
g	KDB-10 HF:DMF=1:10, j = 15 $\frac{mA}{cm^2}$, t=240 min, macroporous.
h	KDB 10,HF:$CH_3CH(OH)CH_3$:H_2O, j = 20 $\frac{mA}{cm^2}$, t=240 min, microporous.
i	Monocrystalline silicon.

Figure 13—Test samples on a holder placed in the SEM

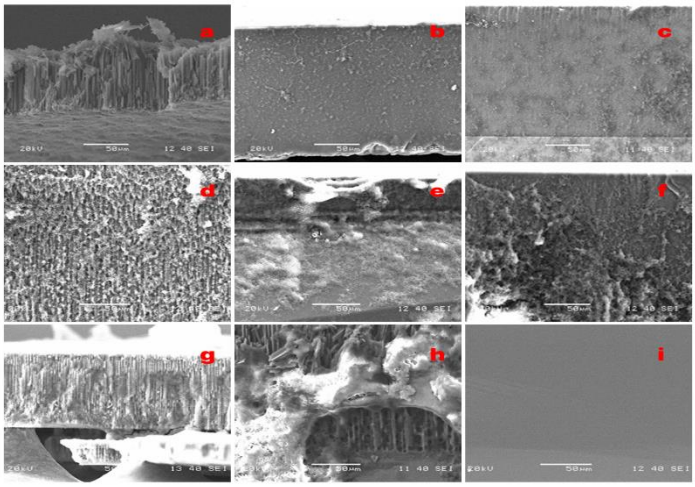


Figure 14 - SEM images of samples after 24 hours of exposure to suspensions (see table 4)

2.5 SEM analysis of samples 14 days after exposure to suspensions

Following the initial image analysis, samples were placed back into their respective suspensions for subsequent structural analysis after 14 days.

During the repeated analysis of the stale cleavage, no decomposition or degradation of the structure was detected, and no changes in the structure and pores relative to the images after a day of exposure were recorded. Only an increase in the biomass of the formed films of the model medium on the samples was recorded (Images a–i) [53].

The images below confirm the absence of degradation of the structure of porous silicon with different pore morphologies during long-term contact with the bacterial environment and suggest that any of the studied coatings are suitable for use in MFCs with proton exchange membranes over a period of 14 days.

However, it was decided during the last iteration of the sample analysis to analyze the fresh chips to obtain a more detailed picture of the changes in the structure of the samples in the pore volume.

Figure 15 - SEM images of samples after 14 days of exposure to suspensions (see table 5)

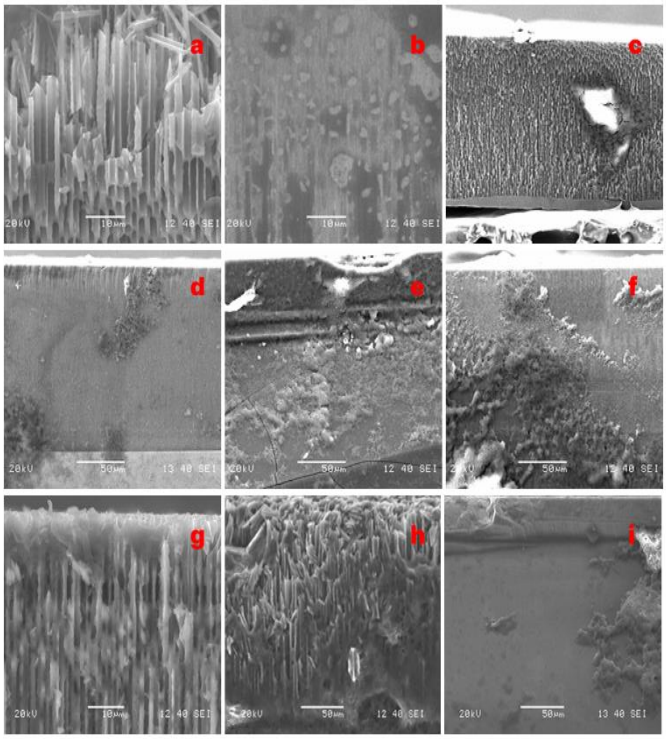


Table 5 - Labels for SEM images of samples after 14 days of exposure to suspensions

Images	Description
a	KDB 9, HF:DMF=1:10, t=300 min, j = 15 $\frac{mA}{cm^2}$, macroporous.
b	KDB 0.005, HF: C_2H_5OH=1:1, j = 100 $\frac{mA}{cm^2}$, t=50 min, nanoporous.
c	KDB 0.002 HF: C_2H_5OH =1:1+STAC, j = 80 $\frac{mA}{cm^2}$, t=60 min, nanoporous.
d	KDB 1000 HF:$CH_3CH(OH)CH_3$=6:1+STAC, j = 10 $\frac{mA}{cm^2}$, t=300 min, HPC-var.
e	KDB-10 HF:DMF=1:6, j = 15 $\frac{mA}{cm^2}$, t=240 min, nanoporous.
f	KDB 10, HF:$CH_3CH(OH)CH_3$ = 6:1, j = 20 $\frac{mA}{cm^2}$, t=150 min, HPC-var.
g	KDB-10 HF:DMF=1:10, j = 15 $\frac{mA}{cm^2}$, t=240 min, macroporous.
h	KDB 10,HF:$CH_3CH(OH)CH_3$:H2O, j = 20 $\frac{mA}{cm^2}$, t=240 min, microporous.
i	Monocrystalline silicon.

2.6 SEM analysis of samples after a month of exposure to suspensions

After the initial image analysis, the samples were placed back into the appropriate suspensions for subsequent analysis of their structure on a fresh cleavage section after one month.

When analyzing fresh cleavage, no decomposition or degradation of the structure was detected, and no changes in the structure and pores relative to the images after a day of exposure were recorded. Moreover, when analyzing fresh cleavage, the absence of biofouling in the pore volume was noted, which confirmed the absence of clogging of the porous silicon pores when bacteria were placed on the surface of the sample and confirmed the ability of porous silicon to place bacteria on the surface and simultaneously prevent membrane contamination.

The images below confirm the absence of degradation of the structure of porous silicon with different pore morphologies during long-term contact with the bacterial environment and suggest that any of the studied coatings are suitable for use in MFCs with a proton exchange membrane for a period of one month.

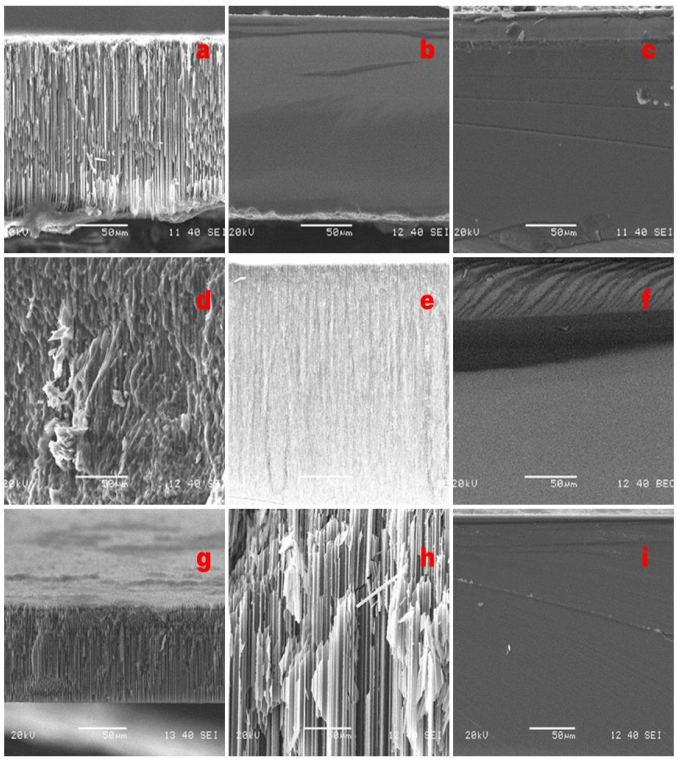


Figure 16 - SEM images of samples after a month of exposure to suspensions (see table 6)

Table 6 - Labels for SEM images of samples after a month of exposure to suspensions

Images	Description
a	KDB 9, HF:DMF=1:10, t=300 min, j = 15 $\frac{mA}{cm^2}$, macroporous.
b	KDB 0.005, HF: C_2H_5OH=1:1, j = 100 $\frac{mA}{cm^2}$, t=50 min, nanoporous.
c	KDB 0.002 HF:C_2H_5OH=1:1+STAC, j = 80 $\frac{mA}{cm^2}$, t=60 min, nanoporous.
d	KDB 1000 HF:$CH_3CH(OH)CH_3$=6:1+STAC, j = 10 $\frac{mA}{cm^2}$, t=300 min, GPC-var.
e	KDB-10 HF:DMF=1:6, j = 15 $\frac{mA}{cm^2}$, t=240 min, nanoporous.
f	KDB 10, HF:$CH_3CH(OH)CH_3$ = 6:1, j = 20 $\frac{mA}{cm^2}$, t=150 min, HPC-var.
g	KDB-10 HF:DMF=1:10, j = 15 $\frac{mA}{cm^2}$, t=240 min, macroporous.
h	KDB 10,HF:$CH_3CH(OH)CH_3$:H_2O, j = 20 $\frac{mA}{cm^2}$, t=240 min, microporous.
i	Monocrystalline silicon.

2.7 Growing a culture of *Bacillus subtilis 534* bacteria in contact with the samples being studied

The pH values of the suspensions obtained in contact with the samples under study (Table 3) confirmed the possibility of growing bacteria in contact with porous silicon samples with different pore structures and morphologies [53] [58]. However, to confirm the compatibility of silicon with bacteria, a colony of Bacillus subtilis 534 bacteria was grown in contact with the samples under study.

B. subtilis can tolerate extreme environmental conditions and is considered the best studied Gram-positive bacterium [54]. *Bacillus subtilis* is an electrochemically active culture used in MFC systems, with an observed power generation efficiency of 270 mW/ m^2 [55].

2.8 Conducting the experiment

To prepare the nutrient medium, L-broth was used, which included yeast extract (5 g/l, enzymatic peptone (15 g/l, sodium chloride (5 g/l, agarose (17 g/l, and distilled water (1 l [56]. g, respectively, because it was sufficient to prepare 50 ml of the solution for the experiment. Suspensions with 0.25, 0.75, 0.25 g and 0.85 g, respectively.

The component suspensions and distilled water were then placed in conical flasks and thoroughly mixed. The flask was then placed on an ES-H4040 electric heating plate (Figure 17) to melt the solid components and obtain a clear solution. The solution was periodically stirred during boiling for 20 min.

Figure 17 - Drawing-heating plate ES-H4040

After melting all components, the solution was cooled to 50 °C. After cooling the solution, the prepared nutrient medium was poured into two Petri dishes, which were thoroughly washed and sterilized in a drying oven at a temperature of 180

°C for 1.5 hours [56] (25 ml per dish) for solidification and formation of a solid nutrient medium.

"Liquid Sporobacterin" containing *Bacillus subtillis 534* was used as a source of bacterial culture. A suspension of the agent in the amount of 200 µL for the first Petri dish and in the amount of 500 µL for the second Petri dish was preliminarily poured into glass beakers sterilized with an alcohol lamp flame, from which it was then applied to solid nutrient media with a glass rod sterilized with an alcohol lamp flame.

Next, as a reference sample, bactericidal patches were placed in both dishes to create a zone of bacterial growth inhibition around itself. After this, pre-cleaned and sterilized in a drying cabinet at a temperature of 180°C for 1.5 hours [56], then samples (Figure 18) were placed in the first Petri dish at the same distance from the walls of the dish and from each other with the etched side facing the nutrient medium. Then, the Petri dishes containing the samples placed in them were loaded into a drying cabinet, where they were thermostatted at 30 °C for 24 h to incubate the bacteria [52].

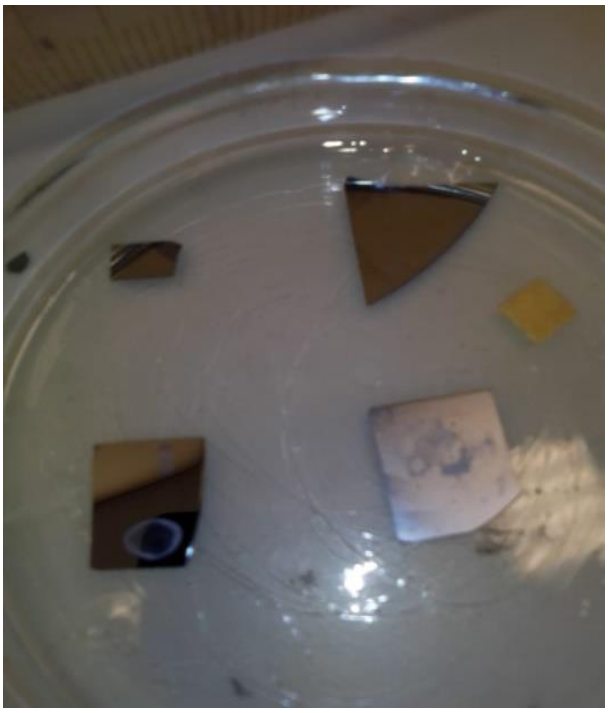


Figure 18 - Petri dish with 4 samples under study

3 Results and Discussion

3.1 Introduction

This section describes the experimental settings that contributed to the results. As a result, comparisons and challenges must be overcome to achieve the desired outcomes and objectives.

The first part of the experiment was to pre-wash the samples in alcohol and to study their different pore morphologies (microporous, macroporous, nanoporous, GPC/HPC variants), the reference bacterial medium (*Lactobacillus Acidophilus and Streptococcus thermophilus*) were prepared from Acidophilin Bakzdrav starter culture. To further examine the biocompatibility of porous silicon in this study, a stronger bacterium, *Bacillus subtilis*, was used.

Ascertaining the non-toxicity of porous silicon is important, and the fermentation outcomes for each suspension were obtained. To ascertain the biocompatibility of porous silicon and its capacity to support bacteria on its surface, the samples were examined for 24 h, 14 d, and a month after the pH values for each suspension were determined.

3.2 Experimental results

Petri dishes were removed from the oven for evaluation after incubation. The experiment in Petri dish 1 (Figure 19) was unsuccessful because the reference sample of the bactericidal patch did not result in the formation of an area of growth inhibition surrounding itself, even though there were no growth inhibition zones surrounding the test samples. The greater transparency and smaller bacterial colonies in Petri dish 1 (Figure 19) compared to Petri dish 2 (Figure 20) indicate that only a relatively small amount of bacteria was introduced into the nutrient medium.

Figure 19 – Image showing the failed experiment for Petri dish 1

As demonstrated by the development of a growth inhibition region surrounding the reference bactericidal patch and the lack of such surrounding the porous silicon samples, the experiment was successful in Petri dish 2 because a greater quantity of *Bacillus subtilis* was added [57]. This further demonstrates the biocompatibility of porous Si and its capacity to support bacteria on its surface.

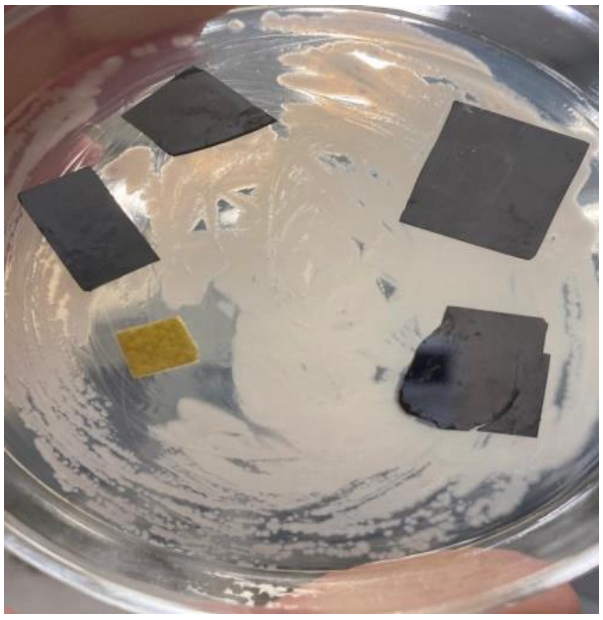


Figure 20 – Image showing a successful experiment for Petri dish 2

3.3 Attempt to measure the pH of the second Petri dish medium

Attempts were made to test the pH of the medium obtained for the second Petri dish to validate the experiment's success. A litmus indicator (Figure 21), methyl orange (Figure 22), and bromophenol blue (Figure 22) were used to determine the pH of the medium because the available pH meter is unsuitable for use in solid and semi-solid media. No color change occurred in any of the instances, which may be because the subsequent suspension was consistent.

Figure 21 – Image showing no color change when litmus indicator was used

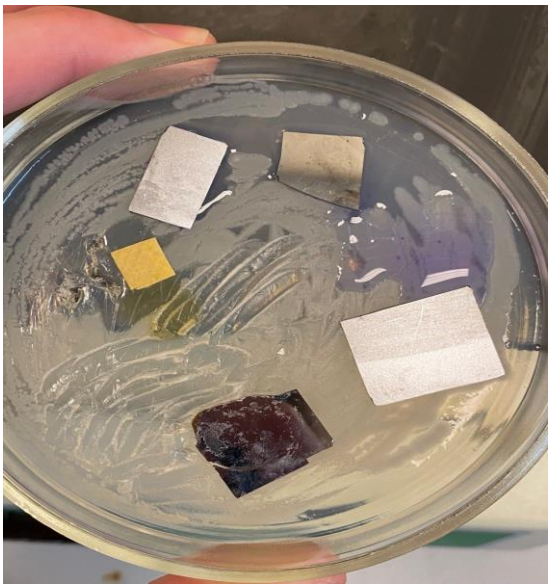


Figure 22 - Image showing no color change when methyl orange (yellow spot) and when bromophenol blue (blue spot) were used

PSi samples showed very good biocompatibility with all tested bacterial strains, and SEM images revealed stable biofilm attachment with all the morphologies of PSi studied. Long-term exposure from 24 h to one month showed no pore clogging or structural degradation. The acidic pH also confirmed that fermentation was successful, and microbial activity was encouraged to take place. An experiment with *Bacillus subtilis* showed that PSi is not toxic and that it is a reliable material that supports the growth of biofilms, which demonstrates its potential for use in microbial fuel cells.

CONCLUSION

Porous silicon (PSi) has been shown in this study to be a suitable anode material for sustainable microbial fuel cells (MFCs). The study demonstrated that PSi has outstanding biocompatibility, structural resilience, and strong resistance to biofouling across a range of pore morphologies (macroporous, nanoporous, and GPC/HPC) through methodical experimentation

with *Lactobacillus acidophilus, Streptococcus thermophilus*, and *Bacillus subtilis*.

The long-term stability of PSi in biologically active conditions was highlighted by SEM analyses conducted after 24 h, 14 d, and 30 d of exposure, which showed steady biofilm growth without pore clogging or structural deterioration. *Bacillus subtilis* trials confirmed PSi's potential of PSi to promote microbial growth and electron transfer, while also confirming its non-toxic nature and suitability for electroactive settings.

All of the investigated porous silicon (PSi) samples, irrespective of pore morphology, showed outstanding biocompatibility, structural integrity, and resistance to biofouling over an extended period of microbial exposure, according to the experimental results, confirming their general suitability for use as anode materials in microbial fuel cells (MFCs). However, the nanoporous and hierarchical pore column (HPC-var) structures demonstrated improved bacterial adhesion and biofilm production among the different morphologies studied, suggesting their potential as promising anode materials.

Through the resolution of significant issues with existing MFC technologies, including biofouling and electrode degradation, this study established porous silicon as a practical, affordable, and expandable substitute for conventional carbon-based electrodes. Its significance in the search for decentralized and environmentally friendly energy solutions is further supported by its capacity to increase power output, prolong operating lifetimes, and facilitate the production of sustainable energy from organic waste, making it a good alternative to fossil fuels such as solar energy.

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