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Author's Affiliation:
University of Baghdad – Iraq

***Corresponding Author:**
Zainab Ziad Ismail
Email: dr.zainab.zead@coeng.uobaghdad.edu.iq

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Photosynthesis microbial desalination cell: Analysis and kinetic study of microbial community contribute to biofilm formation, system performance and bioenergy recovery

Ahmed Mustafa Sadeq, Zainab Ziad Ismail*

Abstract

Background: Detection of bacterial species using 16S rRNA is a popular approach in microbiology. This method focuses on 16S rRNA gene which includes both conserved regions shared among bacterial species and variable regions unique to each species. This study aimed for the first time to apply this technique for identifying and classifying the bacterial species which contribute to the formation of anodic biofilm in a tubular photosynthetic microbial desalination cell (PMDC).

Methods: A tubular photosynthesis microbial desalination cell was designed and set up for simultaneous wastewater biotreatment and desalination of seawater associated with clean power generation.16S rRNA sequencing was used for characterization of the dominant microbial strains in the anodic biofilm. The materials involve DNA extracting from bacteria and PCR amplifying for 16S rRNA gene. The kinetic of the bacterial growth in relation to the substrate utilization was studied.

Results: The results revealed the identification of 19 new dominant microbial strains; 13 in the initial shallow biofilm and 6 in the developed biofilm. Results of evaluating the PMDC performance demonstrated that maximum removal efficiency of organic content from sewage was 93±3% associated with power generation of 24.3±2.5 mW/m^3 and 70±4% desalination efficiency of saline water. Results of the kinetic study of biomass growth demonstrated that among the 5 examined models, Monod and Blackman models significantly fitted the experimental data with determination coefficients (R^2) of 0.951 and 0.907, respectively.

Conclusion: This study adds to our knowledge of the anode biofilm's involvement in PMDC performance by identifying dominant microbial strains using 16S rRNA sequencing. The findings emphasize microbial contributions to simultaneous treatment of wastewater, desalination of sea water, and electricity generation. This experimental and theoretical investigation paves the way for future breakthroughs in microbial desalination technology, addressing crucial water scarcity issues.

Introduction

The bioelectrochemical system (BES) is an important technique that uses exoelectrogenic bacteria for biodegradation of organic matters to generate green energy and other value-added products from waste and wastewater [1]. Microbial desalination cell (MDC) as an environmentally friendly technique is considered as a type of the BES system. MDC generates electricity in addition to desalination of brackish or saline water simultaneously with wastewater treatment [2]. For elimination of the toxicity and cost issues associated with the use of chemical electrolytes and noble catalysts in MDCs, photosynthetic microbial desalination cells (PMDCs) which contain the biocathodes of photosynthetic microorganisms were suggested as a potential alternative to MDCs containing abiotic cathodes [3].

On the other hand, for the development of efficient biotreatment, it is necessary to determine the microbiota contents in wastewater and characterization of the strains that metabolize organic matters [4]. Culturing of microbial species is used for characterization based on metabolism; this method identifies microbes which grow in culture only. Most species in activated sludge is difficult to grown in culture [5]. So, most microbiologists strove to understand the diversity and organization of microbial communities. Around 1985, utilization of molecular biology tools was started and changed the outlook for microbial ecologists. PCR and DNA can be used to amplify specific DNA selectively and precisely, allowing for direct investigation of genetic data from individual microbes as well as entire communities. The 16S rRNA (small subunit ribosomal RNA) was the first target for this method and is still a popular choice for genetic investigation in prokaryotes. This rRNA offers information regarding the phylogenetic identity of microbes, so answering the first fundamental question of microbial ecology. [6]. For both bacterial classification, sequencing of the 16S rRNA genes has been used generally as an important identification tool due to its presence in almost all types of bacteria [7]. Its function doesn't change with time, and the 16S rRNA gene (1500 bp) is quite big to allow the identification of genus and species of isolates [8]. Understanding and simulating the bacterial growth in the microbial fuel cells (MFCs) are essential for the optimization and design of these cell types.

Several previously reported studies concerned of the microbiological and biochemical characterization of sewage sludge from the organized sector. However, limited studies are available concerning characterization of microbial community for biofilm formation, specifically in bioelectrochemical systems (BES). Cheng et al., [9] analyzed 63 samples of sludge and 79 samples of biofilm from 13 anaerobic membrane bioreactors to identify and characterize the predominant microbial community. The data suggested that the core microbial species found in at least 90% of sludge and biofilm samples could have a major impact on bioreactor performance. Pan et al., [10] examined the microbial population using DNA extraction, PCR amplification, and Illumina sequencing to amplify the V3-V4 region of 16S rRNA using PCR (ABI GeneAMP®9700). Zhao et al., [11] characterized and analyzed the microbial community in MFC-DBR reactor. Genomic DNA was extracted from the samples using CTAB/SDS method and were utilized for amplifying V4 region of the 16S rRNA gene. Products of the polymerase chain reaction (PCR) were detected using 2% agarose gel electrophoresis. Maurya et al., [12] isolated *Bacillus vallismortis* and other bacteria from tannery sludge and tested for biofilm development in various environments. Zhao and Zhao [13] studied microbial strains as they formed varied thicknesses of anodic biofilm in a microbial fuel cell powered by wastewater containing hydrolyzed polyacrylamide (HPAM). To begin biofilm formation, active sludge samples were introduced into the fuel cell. DNA extraction from biofilm samples was followed by PCR amplification of genomic DNA with universal primers. Sequencing was then performed in accordance with a conventional methodology.

Wu et al., [14] analyzed the microbial community for chloramphenicol-degrading anode biofilm formation in microbial fuel cells. Zhu et al., [15] studied the structural pattern of microbial community of anodic biofilm at various steps of formation to gain insight on the change of *Geobacter* enrichment in a double-chambered MFC. DNA extraction of the biofilm samples was done using a rapid DNA spin kit. PCR was used to amplify the hypervariable V3-V4 sections of the bacterial 16S rRNA gene using polymerase chain reaction (PCR). So far, to the authors knowledge, none of the previously reported studies considered the microbial community analysis and characterization of the dominant new strains in the anode biofilm in photosynthetic microbial desalination cell (PMDC).

The principal objectives of this study were; (1) identify and characterize for the first time the microbial community of the anode biofilm in a photosynthetic microbial desalination cell (PMDC) and evaluate their contribution in the anode biofilm formation, (2) investigate the effect of the newly characterized microbial strains on the general performance of the PMDC including organic content removal from sewage, desalination of saline water associated with bioenergy recovery, and (3) adopt different kinetic models to describe the bacterial growth process within the anodic biofilm texture.

Methods

Biocatalyst

Activated sludge samples were collected from an aeration basin at a local sewage treatment plant in Baghdad, Iraq. The samples were taken throughout the spring season, specifically in March, at temperatures ranging from 22 to 25°C. The pH of the sludge samples ranged from 5.8 to 7.4. The collected samples were conserved and kept at 4°C until needed to start the anodic biofilm in the PMDC.

Construction and setup of the PMDC

A cylindrical-shaped photosynthetic microbial desalination cell (PMDC) of 13 cm internal diameter was designed, constructed and installed in this study as shown in Fig.1. The PMDC was fabricated of transparent acrylic material. It consisted of 3 chambers which were; the anaerobic bioanode chamber, the desalination mid chamber, and the aerobic biocathode chamber. A cation exchange membrane (CEM) was employed to segregate the biocathodic chamber from the desalination chamber, while an anion exchange membrane (AEM) was utilized between the bioanode and desalination chambers. Both types of membranes were provided by (CMI 7000, Membranes international). The bioanode compartment was fueled with 2250 ml of actual sewage with temperatures ranging from 25°C to 30°C, and pH levels varying between 6.5 to 8 which was gathered from the major inlet pipe to a local sewage treatment plant in Baghdad, Iraq. The organic content concentration in the sewage was 800 ± 40 mg/L as COD. The desalination compartment contained 1250 ml of actual saline water collected from Shatt Al-Arab River, southern Iraq. The average concentration of the total dissolved solids (TDS) in saline water was 10000 ± 100 mg/L. The biocathode compartment was fueled with 2250 ml of microalgae suspension (bio-catholyte), respectively.

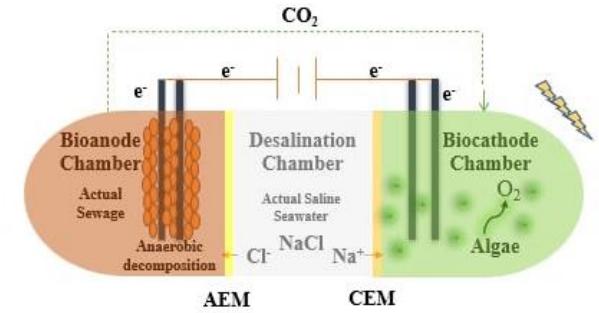

Figure 1: Schematic diagram of PMDC The anode generates electrons, which traverse an external circuit to the cathode, aiding reduction reactions by transferring to electron acceptors. A mid-chamber, with saltwater, separates the anode and cathode. Ions from this chamber move through cation and anion exchange membranes to respective compartments.

Isolation of the dominant microorganisms

Two groups of biomass samples named G1 and G2 were subjected to and underwent the molecular identification test to characterize and identify the dominant species and strains in the anode biofilm in the suggested PMDC. G1 included the group of biomass samples picked up from the initially initiated biofilm on the anode electrodes. G2 involved the group of biomass samples obtained from the developed grown biofilm by the end of 144 h continuous operation of the PMDC system.

Four different bacterial culture mediums (McConkey agar, blood agar, Mueller Hinton Agar, and brain-heart infusion) were individually prepared according to the procedures outlined by Ifeanyi et al., [16]. The media were prepared according to the standard preparation protocols for each type. Bacteria were isolated from stored sludge samples using blood and MacConkey agar media. These plates were incubated overnight at 37°C [4].

DNA Extraction Methods for Bacterial Identification

DNA was extracted from bacteria according to the manufacturer's instructions ABIO Pure™ Total DNA extraction, USA. A single colony was vaccinated with nutrient broth and then grown at 37°C for 24 hours. After extraction the DNA concentration was measured using a Quantus Fluorometer (Promega, USA) to assess sample quality for downstream applications [17].

Amplification of 16S rRNA gene sequencing

The primer utilized in this study for the PCR reaction was produced by Macrogen Inc for genetic analysis (Korea). The universal primers including the forward primer 27F (AGAGTTGATCMTGGCTCAG), and the reverse primer 1492R (TACGGYTACCTTGTTACGACTT) were utilized [18]. The 16 S rRNA gene fragment was amplified with a Thermo Fisher Scientific PCR Thermal Cycler (USA). To perform the PCR reaction, add 12.5 µl of Master Mix, 2 µl of DNA, 1 µl of forward and reverse primers, and 7.5 µl of deionized water to a 25 µl volume [17]. After enhancement by PCR, agarose gel electrophoresis was adopted to confirm the presence of amplification, after that the PCR product was sent to Macrogen Company for sequencing analysis.

Monitoring of bacterial growth

The growth of the microorganisms was monitored during the development progress of the anodic biofilm in the PMDC by determining adenosine triphosphate (ATP) using the BacTiter-Glo™ Microbial Cell Viability Assay (Promega, USA). This test approach is a method for assessing the numeral of viable bacterial cells in a culture based on the ATP quantity. Also, the residual organic content represented by the (COD) treated effluent was measured on a daily basis to assess the

biodegradation efficiency of COD over the time of operation. The biodegradation potential is an indicator of the biomass growth.

Kinetic models for bacterial growth

Monod, a French biologist, researched the development of bacteria cultures and the parallelism with the Michaelis-Menten theory in 1940, and proposed, therefore, a non-linear relationship between the rate of specific growth and substrate concentration.

According to the Monod kinetic model [19], the rate of specific growth increases significantly for low substrate concentrations and slowly for large substrate concentrations, until bacterial saturation is achieved. This limit refers to the greatest specific growth rate (μ_{max}). The Monod-constant, K_s, is the concentration of substrate at half the maximum specific growth rate ($\frac{\mu_{max}}{2}$). Pfeffer [20] observed that Monod model cannot be used to represent the biodegradation of household garbage as a complex mixed substrate. Five kinetic models which may comprehensively explain biochemical reactions were adopted to investigate the most convenient model for the target case. Blackman model and Tessier model are another form of kinetic model [21,22,23], Tessier model is also renowned as the exponential model, which describes the specific growth rate as a function of continuous substrate concentration.

Han–Levenspiel suggested another type of kinetic model as an extended Monod model. Han and Levenspiel [25] and Okpokwasili and Nweke [26] reported that Han–Levenspiel is efficient in describing the microorganism growth at various substrate concentrations by considering a generalized nonlinear equation to account for the effect of toxicity generation during the substrate biodegradation.

In 1958, Herman Moser introduced for the first time another kinetic model termed as Moser model. It is an extension of the Monod model by introducing (n) into Monod model [24].

In MFC system, the μ and the μ_{max} normally substitute as the substrate degradation rate (r) and the maximum substrate degradation rate (r_{max}) and usually measures in term of (kg/m^3. d) or substitutes as the power or power density and the maximum power (mW) or power density (mW/m^3) [23, 27]. The kinetic parameters were estimated to use MATLAB software and excel solver tools.

Results

The performance evaluation of the suggested (PMDC) involved comprehensive assessments of its functionality. Three key parameters were analyzed: organic content removal from raw sewage, total dissolved solids (TDS) elimination from saline water, and power generation. Fig.2 presents the profiles of organic content removal efficiency as (COD) in raw sewage, TDS elimination efficiency in saline water, and power generation.

The PMDC had a COD removal efficiency of 93±3%, suggesting its effectiveness in lowering organic content in raw sewage. The PMDC eliminated TDS with 70±4% efficiency, making it a feasible choice for saline water treatment. Moreover, the PMDC system exhibited remarkable power generation capabilities, generating an average of 24.3±2.5 mW/m^3, which adds to its practical uses.

In addition, the study investigated the characterization of bacterial colonies in the anode biofilm before (G1) and after (G2) PMDC operation.

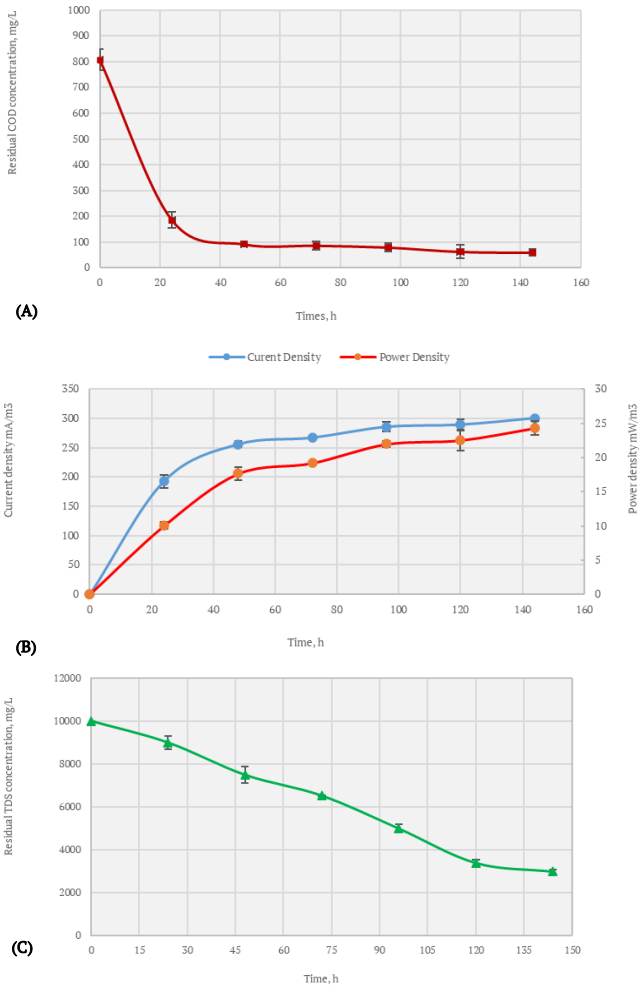

Figure 2: The performance of PMDC; (A) Represents the relationship between COD removal efficiency and time, showcasing the effectiveness of the PMDC in wastewater treatment. (B) Depicts the power generation capacity of the PMDC over time, demonstrating its capability to generate electricity through electrochemical reactions. (C) Displays the reduction in Total Dissolved Solids (TDS) concentration over time, indicating the desalination capability of the PMDC.

Molecular identification revealed 13 new strains in the initial biofilm (G1) and 6 new strains in the developing biofilm (G2), as shown in Table 1. These strains fell into several groups, such as cocci, gram-negative rods, and coccobacilli. Two prominent bacteria in the PMDC system, *Escherichia coli* and *Staphylococcus haemolyticus*, are noteworthy for their persistence in both biofilm stages.

The results of characterization confirmed the existence of 13 and 6 new strains in the anodic biofilm before and after the PMDC operation was shown respectively in Table 1.

Additionally, the study explored the kinetics of microorganisms' growth in the anode biofilm. ATP (adenosine triphosphate) was monitored at predetermined intervals, and it was observed that ATP increased as COD decreased, indicating an inverse relationship between substrate consumption and microorganisms' growth (Fig.3).

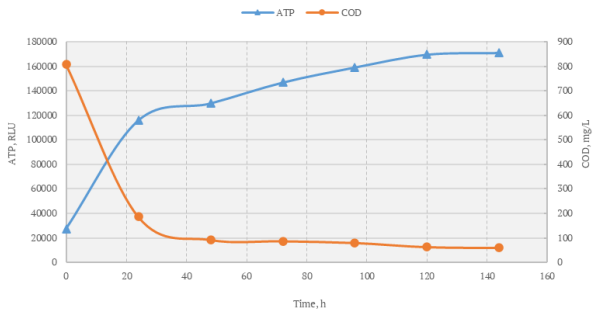

Figure 3: illustrates the relationship between cells' growth, measured as adenosine triphosphate (ATP) production, and substrate consumption, represented by chemical oxygen demand (COD), over time. As time progresses, the consumption of substrate correlates with the growth of cells, reflected by ATP production.

Furthermore, five alternative kinetic models were used to estimate the specific growth rate of mixed bacterial cells (Fig. 4). The Monod model, characterized by a maximum specific growth rate (μ_{max}) of 6.094 per hour and a half-saturation constant (K_s) of 630 mg/L, demonstrates a good fit with an R-squared (R^2) value of 0.951. Similarly, the Blackman model exhibits a μmax of 6.283 per hour and a K_s of 313.7 mg/L, achieving an R^2 of 0.907. The Tessier model, with a μmax of 6.725 per hour and a K_s of 350 mg/L, shows an R^2 of 0.854. The Moser model, more complex with additional parameters, including empirical constant (m) with a value of 0.978, has a μmax of 7.102 per hour and a K_s of 480 mg/L, yielding an R^2 of 0.865. Lastly, the Han-Levenspiel model, indicating empirical constant (n = 1, m = 1.1055) and a threshold substrate concentration (S_m) of 750 mg/L, has a μmax of 6.998 per hour and a K_s of 430 mg/L, but notably, it exhibits a lower R^2 value of 0.221, suggesting a less accurate fit compared to the other models.

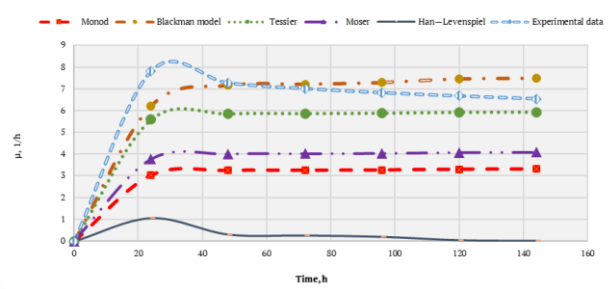

Figure 4: Compares the experimental results of the specific growth rate for mixed bacterial cells to the values predicted by the five adopted kinetic models.

Discussion

The PMDC performance results show promise in terms of organic content removal, TDS elimination and power generation. With COD removal effectiveness of 93±3% the system successfully eliminates organic pollutants from raw sewage and this is critical for wastewater treatment. The significant elimination efficiency implies that the PMDC's microbial community effectively degraded organic pollutants via multiple metabolic activities, improving overall system performance [28,29].

Desalination of sea water using the PMDC is effective with a 70 ± 4% TDS removal effectiveness, which makes it possibly one effective solution to the challenges of water inadequacy. The TDS reduction can be associated with the combined anode side electrochemical reaction and microbial metabolism. Furthermore, the measured 24.3 ± 2.5 mW/m^3 power generation shows that the PMDC can treat wastewater and generate electricity simultaneously. The metabolic activities of microorganisms involved in organic material oxidation and electron transfer generate an electric current in the anode [30]. The analysis of biofilm colonies in PMDC yields valuable information on how microbial populations change over time. The presence of 13 strains in the initial biofilm G1 and 6 new strains in the final biofilm G2 proves the PMDC capability of supporting a large range of microbial communities [31].

Different bacterial groups within the PMDC could be further identified by using molecular techniques. Most bacterial species have different 16S rRNA gene sequences demonstrating the gene's value as a genetic marker for bacterial identification. All these results demonstrate that 16S rRNA gene sequencing is a reliable and powerful tool for bacterial detection [4, 32, 33]. Using the use of this molecular approach, we can better understand the microbial populations in PMDCs and other wastewater treatment systems, enabling us

to make the most of these technologies for the long-term treatment of water and production of energy.

The kinetic modeling results showed insight into microbial growth dynamics within the anode biofilm. This reinforces the fact that, since the match of the experimental data to the Monod and Blackman models is very effective, it represents substrate utilization and microbial growth as biological kinetics, establishing the applicability of the models in the prediction of microbial behavior within the PMDC system [34]. However, the poor fitting of the Han-Levenspiel model underscores the need for more advanced and specialized models to capture the intricate interactions within the microbial community and their reaction to environmental changes.

At last, the PMDC system's performance evaluation and microbiological characterization revealed potential applications in wastewater treatment and desalination. The research improves knowledge in the field of microbial electrochemical technologies and sets the foundation for future optimization and scaling-up of PMDC systems for sustainable water treatment and energy production.

In this study, characterization and identification of the dominant active microbial strains contribute to the anode biofilm formation in a tubular PMDC by using 16S rRNA sequencing demonstrated 19 new dominant microbial strains; 13 in the initial shallow anode biofilm, and 6 in the developed biofilm. Excellent removal efficiency of organic content associated with bioenergy recovery and adequate desalination of brackish water indicated the effectiveness of the new identified microbial strains for bioelectrochemical treatment process and the general performance of the PMDC. Kinetic study of the bacterial growth process revealed that Monod and Blackman models significantly fitted the experimental data.

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Author Contributions

Ahmed M. Sadeq: carried out experimental work, developed the models, performed the preliminary data analysis, and wrote the first draft of paper.

Zainab Z. Ismail: supervised and directed the project, designed the experimental tests, carried out the final data analysis, and prepared the final version of the paper.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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