

Development of Integrated Photobioelectrochemical System (IPB): Processes, Modeling and Applications

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ABSTRACT (academic)

Effective wastewater treatment is needed to reduce the water pollution problem. However, massive energy is consumed in wastewater treatment, required to design an innovative system to reduce the energy consumption to solve the energy crisis. Integrated photobioelectrochemical system (IPB) is a powerful system to combine microbial fuel cells (MFCs) and algal bioreactor together. This system has good performance on the organic degradation, removal of nitrogen and phosphorus, and recover the bioenergy via electricity generation and algal harvesting. This dissertation is divided to twelve chapters, about various aspects of the working mechanisms and actual application of IPB. Chapter 1 generally introduces the working mechanisms of MFCs, algal bioreactor, and modeling. Chapter 2 demonstrates the improvement of cathode material to improve the structure and catalytic performance to improve the MFC performance. Chapter 3 describes the process to use microbial electrolysis cell (MEC) to generate biohydrogen for the energy recovery. Chapters 4 and 5 demonstrate the application of stable isotope probing to study *Shewanella oneidensis* MR-1 in the MFCs. Chapters 6 to 8 describe the application of models to optimize MFC and IPB system performance. Chapter 9 describes the strategy improvement for the algal harvesting in IPB. Chapter 10 describes the application of scale-up bioelectrochemical systems on the long-term wastewater treatment. Chapter 11 finally concludes the perspectives of IPBs in the wastewater treatment and energy recovery. This dissertation comprehensively introduces IPB systems in the energy recovery and sustainable wastewater treatment in the future.

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ABSTRACT (public)

The resource of pure water becomes more and more valuable, and the large discharge of the wastewater into the environment would even cause the environmental pollution. Thus, the wastewater is a necessary method to remove the organics out of the wastewater. However, the large energy consumption is a critical issue to solve due to the global energy burden. How to reduce the energy consumption in the wastewater treatment is the required step to achieve the sustainable water treatment. Integrated photobioelectrochemical system (IPB) is a new promising technology, alternative to the traditional wastewater treatment techniques (e.g., anaerobic digester or activated sludge reactor) with low energy consumption. The IPB system was to combine microbial fuel cells (MFCs), which is a typical bioelectrochemical system (BES), and the algal bioreactor together, to achieve the performance on the organic degradation, removal of nitrogen and phosphorus in the wastewater, and recover the bioenergy via electricity generation and algal harvesting. The system was proved to be effective, but most of the IPB systems were only proved to work in the laboratories, and there is still a large potential space to improve the IPB system performance in the actual environment. Herein, this dissertation combines multiple studies about the IPB improvement and scaled-up process in the real wastewater treatment. Chapter 1 generally introduces what are MFCs, algal bioreactor and modeling simulations. Chapter 2 demonstrates the method about how to improve the MFC material to enhance the treatment performance for better MFC performance. Chapter 3 describes how to use BES to convert the organics to the renewable gas (e.g., H_2 and CH_4) to recover the energy. Chapters 4 and 5 demonstrate the application of stable isotope probing to study the microbial behavior in the MFC. Chapters 6 to 8 describe the applications of model simulations to optimize MFC and IPB performance. Chapter 9 describes the new reactor to improve the algal harvesting process to obtain more energy from the IPB system. Chapter 10 describes how to use the scale-up IPB system to treat the real wastewater treatment. Chapter 11 finally puts forward some perspectives of IPBs in the wastewater treatment and energy recovery. This dissertation comprehensively gives a big picture about the development of IPB systems in the energy recovery and sustainable wastewater treatment in the future.

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Chapter 1 Introduction

1.1 Overview

Water, nutrient, and energy are required resources for the humans to live and develop on the globe. The strategy to obtain these resources would be consistently needing development for the future life. However, the resource shortage and the growing demand by the population explosion become the critical issue for 21st century [1]. For example, currently, about 30% of the global people (around 2.1 billion) lack access to the safe and available water source or the water supply with the suitable sanitation at the home, especially for the rural areas [1]. For the electricity, it was reported that only 75.2 % and 32.4 % of the population in South Asia and Sub-Saharan Africa have the access to the electricity. For Asia, huge constraints are placed on the economic growth due to the huge demand of the electricity and insufficient supply [2]. The energy shortage confines people's life quality and pose pressure on the economic and industrial growth, and the major causes for the energy shortage need to be analyzed to find the direct strategies to solve problems. The major causes include the overconsumption of the stored energy and the growing population for larger resource requirement. More options for the water, nutrient and energy recycling should be explored, and the water energy wasting should be reduced.

In this dissertation, the innovative technologies of microbial fuel cell and algal bioreactor are introduced to achieve the purpose of water cleaning, nutrient recovery and energy generation. Furthermore, the integration of these two technologies is described to show the synergistic relationship of these two technologies together to compensate for the drawbacks of each single component and obtain the best wastewater treatment effect and energy recovery. The innovative integrated system is called integrated photo-bioelectrochemical (IPB) treatment system. The bench-scale and the lab-scale IPB system has been successfully constructed with good performance of organic degradation, nutrient removal and energy recovery [3, 4]. The working principles, configuration, system performance and application perspective would be described in this chapter for your information. The objective of the IPB construction is to study and develop the sustainable energy system to benefit the environmental sustainability in the wastewater treatment.

1.2 Current problems in the global sustainability

Global sustainability is increasingly required and understood as a prerequisite to achieve the human development [5]. To achieve the sustainability, many challenges need to be handled. Among all the challenges, the energy section is probably one of the most important and valuable parts due to its strong economic, environmental and technological relevance. Also, the energy is the basic requirement for the living standard and the human wealth. Thus, energy recovery and technological development would be the important focus of the global sustainability and will be describe with details in this chapter.

1.2.1 Energy crisis due to overconsumption and overpopulation

The increasing energy consumption was mainly due to multiple reasons, and one of the major contributing reason is the overconsumption. The energy crisis comes from the exploitation of various resources including oil, gas and coal for the fuel utilization and electricity generation [6]. The energy crisis results from the overconsumption of the natural resources, and the stress on fossil fuels including petroleum, coal and natural gas is increasing. It was reported that the 78.4% of the global energy demand was attributed to the supply of the fossil fuels (Fig. 1.1) [7], with the consumption rate at 0.001 Mb per second [8]. Thus, the fossil fuel reserve would be the major contributing factor for the energy consumption. Since the fossil fuel is the finite resource, and the hydrocarbon age would finally end. It was expected that the peak oil was reported to occur in 2009 with the production rate of 86 Mbpd, but the oil production was reported to drop to 40 Mbpd by 2050 based on the current consumption [9]. In the future, the fossil fuel would finally be used up.

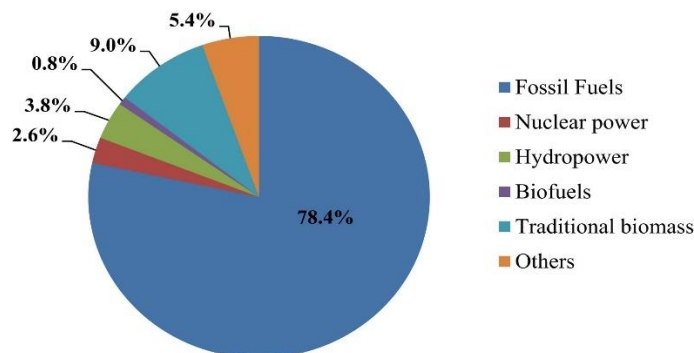


Figure 1.1 World primary energy consumption in 2014.

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The overpopulation problem would be another factor leading to the energy crisis. The high growth rate estimated as 200,000 persons per day [8], results in higher demand of the fossil fuel to increase the depletion rate. Finally, the global population has increased from 1.6 billion in 1900 to 7.2 billion today, and thus the corresponding energy supply has increased from 23 to 548 exajoules (EJ). The estimation of the global population in 2050 modelled by the single logistic function will reach 8.9 billion [10]. This explosion would finally cause an exponential increase in energy demand on limited fossil fuel reserves. For example, higher fuel demand for the automobile vehicles is needed for the public transportation. In 2011, it was estimated that 59% of the fossil fuels was used for the car transportation [11]. The low energy conversion efficiency (at 30 % thermal efficiency) under the current engine technologies exacerbated the fuel overconsumption in the transportation sector [12, 13].

The population explosion and the economic growth also cause higher demand of the electricity to support people's live, and more power plants are needed to convert more fossil fuel to the electricity. It was reported that, in 2010, 17.84 EJ of electricity was consumed in the residential sector, with 39% increase during the last decade [6]. With more residential areas and industrial applications for population growth, the more power plants are needed to meet the rapid increase of the electrical power requirement. For example, in China, about half of the total coal combustion was placed on the electricity generation after rapid urbanization in 2010, with higher proportion share than the average percentage at 39.8% of the coal for the energy supply [14]. Thus, the China's power industry faces huge pressure not only in the emission reduction but also the sustainable energy supply for the residents [15, 16]. The energy shortage and pressure of the energy caused by the overconsumption and overpopulation would pose a long-term restriction for the people's lives in the future, and specifically innovative strategies are needed to solve the problems and recover the energy from the alternative ways to reduce the dependence on the fossil fuel.

1.2.2 Global warming due to carbon emission

The overconsumption of the fossil fuels will produce the greenhouse gas including carbon dioxide (CO₂), nitrogen oxide (NO_x) and sulfur dioxide (SO₂), to cause the global warming and the atmospheric pollution. For example, the atmospheric content of CO₂ has increased year by year, and most proportion came from the power generation and industrial sections (Fig. 1.2).

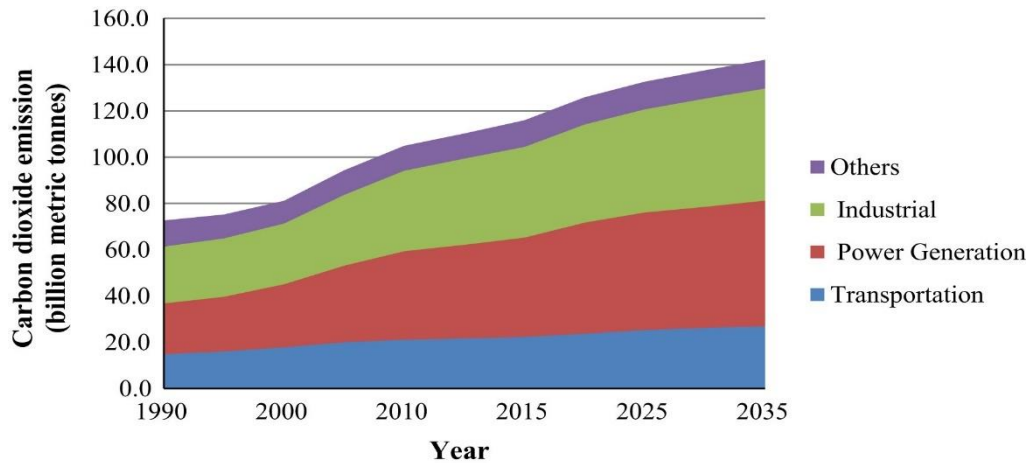


Figure 1.2 Global CO₂ emission from various sections under the time period (1990-2035).

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The rapidly increasing CO₂ emission mainly came from the economic growth from the developing countries, due to the need and propulsion to accelerate the national economy [17]. It was reported that in the last forty years, the gas emission was continuously increasing to finally at 32 billion tons in 2011, due to the greater population and the energy consumption [18]. The global CO₂ emissions was expected to project to 36 billion tons in 2020, and even double the original amount by 2050 [19]. The rapid increase was caused by the expansion of the industrial productivity and resource exploitation will increase the consumption of the fossil fuels. Suitable technologies are needed to reduce the carbon emission and sequester the atmospheric carbon content. Demand reduction of the fossil fuel is a promising direction, and energy alternatives deserves extensive research and exploration to reduce the dependence on the traditional fossil fuels. Thus, an alternatively sustainable fuel source is needed, and a new technology to sequester the greenhouse gas is promising for the future development.

1.2.3 Wasting of energy in wastewater treatment

The huge water consumption and the wastewater production is a severe problem for human life and natural ecosystem in the twentieth century, due to the global overpopulation. Thus, to solve the water scarcity, effective wastewater treatment would be required to reduce the water pollution and recover clean water for the people's daily life. However, massive amounts of energy are required for the wastewater treatment due to the large volume demand [20]. For example, in

America, there are about 14,780 municipal wastewater treatment facilities under the operation in the United States (data derived at October 2014), and thus the average wastewater treatment capacity requirement is about 122 million m³ per day. To guarantee the treatment efficiency and effect, the electricity consumption is massive, equivalent to 3-4 % of the entire national electricity demand [21]. The high electrical requirement to operate the wastewater treatment actually add burden on the greenhouse gas emission [22]. Thus, it is emergent to find a method to achieve sustainability of wastewater treatment to save energy and reduce the carbon footprint [23]. It was reported that the chemical energy potential (dissolved organics) in the wastewater was 2 kWh m⁻³, which was even greater than the energy consumption for wastewater treatment process (0.6-1.0 kWh m⁻³) [24]. Actually, the chemical energy can be viewed as the alternative to the “fossil fuels” existing in the wastewater, and the specific strategy is required to extract and convert the fuel to the usable type. However, there is blank gap in the sustainable technology to convert the chemical energy to the directly applicable energy type (e.g., electricity) that we can use to reduce the energy consumption in the wastewater treatment.

1.3 Technological introduction

To achieve the energy recovery, the chemical energy (organics) in the wastewater deserves focus and intensive research to fill the blank gap for the sustainability. Actually, the wastewater can be considered more as a resource than a waste, and the anaerobic digester is a conventional method to recover the energy in the form of methane (CH₄), mainly applied for the high-strength wastewater. However, the disadvantages of the anaerobic digester are the low energy conversion efficiency, with only about 30-40 % of CH₄ energy converted to the electricity, and the remainder is given off as waste heat [24]. Also, the anaerobic digester is mainly functioned for the high-strength wastewater, and the municipal wastewater (normally low-strength wastewater) may not work efficiently in the anaerobic digester. More efficient technology and related equipment are needed to convert the energy existing in the low-strength wastewater to the electricity. Furthermore, the problem of the high carbon emission also needs to be reduced by using either the alternative fuel instead of the traditional fossil fuels, or the technology to sequester the atmospheric carbon. In the following sections, the technological solutions will be described.

1.3.1 Introduction on the bioelectrochemical systems (BES)

Compared to the anaerobic digester to degrade the high-strength organics under the hydrolysis and fermentation, BES is the innovative technology to degrade the organics with low energy consumption, low sludge production under the temperate conditions, with various configurations with various performances including the electricity generation (microbial fuel cells (MFCs)), hydrogen production (microbial electrolysis cell (MEC)) and desalination (microbial desalination cell (MDC)) [25, 26]. Among all the BES, MFCs are the most basic configuration, in which the electrogenic bacteria in the anode can degrade organic matter to generate electrons, and these produced electrons are transported to the cathode via the external wires. The electrons transported to the cathode have the oxygen as the electron acceptor with the oxygen reduction reaction to complete the electron transportation circuit. Thus, the electricity is finally generated [27]. The typical MFC configuration is the H-shape system, with the anode and the cathode connected together using the cation exchange membrane (CEM) or anion exchange membrane (AEM) to connect two chambers together. The general working mechanism of MFC is shown in Fig. 1.3. The wastewater is fed into the anode chamber, degraded by the anaerobic bacteria attached on the anode electrode material under the anaerobic conditions. The oxidation reaction in the anode can release the electrons from the reductant out to be received by the external resistance to generate the electricity. Then, the released electrons are directed to the cathode along the electrode material and the external wires to the cathode, where the oxygen is supplied as the oxidant during the aeration for the oxygen reduction reaction. To complete the circuit in the MFC for long-term operation, while single-unit electron is released out from the anode to cathode, the solution electroneutrality needs to be maintained to complete the circuit. The functions of the ion exchange membrane (e.g., CEM, AEM) are to allow the ion migration through the membrane to assure the electroneutrality in two chambers, and prevent the substrate or oxygen crossover between the chambers to damage the MFC performance. The driving force for the electron spontaneously flowing from anode (low potential) to the cathode (high potential) is due to the potential differences due to the oxidation-reduction reaction in the anode and cathode, based on Eqs. 1.1 and 1.2, using the acetate and oxygen as the electron donor and acceptor in this case[28].

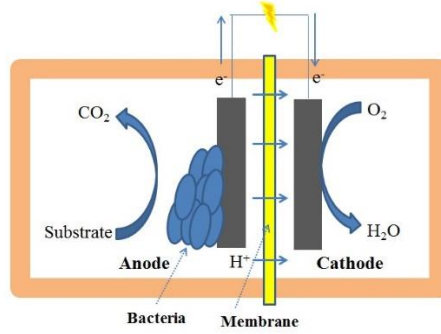
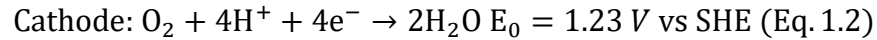
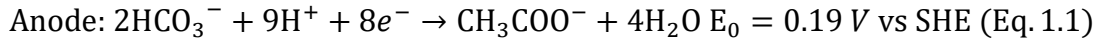


Figure 1.3 The general schematic for H-shape MFCs.



The energy generation is important key factor to be considered about the MFC performance, and the normalized energy recovery (NER) would be the typical indicator to represent the performance for the energy generation. Based on the previous literature review combining 128 MFC publications from 2000 to 2013, it was reported that most MFC could produce NER smaller than 0.3 kWh m^{-3} and about 3.86 kWh kg^{-1} of COD based on treated volume of the wastewater and the removed COD amount [29]. In one MFC study, the system could produce 0.026 kWh m^{-3} while consume 0.024 kWh m^{-3} , indicating that the MFC system could work for the energy recovery[29]. Theoretically, MFC can achieve the positive energy balance if improving the energy extraction strategy from the organics and reducing the energy consumption on the system operation.

MFCs have multiple advantages compared to the traditional anaerobic digester on the energy recovery from the wastewater treatment. First, the MFCs can directly produce the electricity from the wastewater, different from the anaerobic digester needing the methane as the intermediate gas for the electricity generation [30]. It was reported that that 8% of the potential energy was lost during the process of carbohydrate conversion to CH_4 [24]. Furthermore, the CH_4 biogas is difficult to store and transport, and the leaking problem is difficult to avoid and need costly method to store. Ultimately, only 35% of the CH_4 energy is converted to the electricity [24, 31], leading to generally low energy conversion efficiency for the anaerobic digester. Direct conversion from waste to electricity can make the theoretical energy conversion efficiency at 100% for MFCs. Second, the MFCs can generate less sludge than the traditional activated sludge process. It was

reported that the MFCs anode and cathode could remove 85-95% solid content in total [32]. Compared with the TSS in the final effluent at $2214 \pm 314 \text{ mg L}^{-1}$ from the wastewater treatment facility, the TSS from the MFCs was only about $14 \pm 18 \text{ mg L}^{-1}$ [32]. The low sludge production of MFC can reduce the effort and energy consumption for further treatment [33]. Third, the MFC can work in the temperate conditions, different from the anaerobic digester in the thermophilic condition. The bacteria in the anode can serve as the microbial electrocatalysis to degrade the organics in the temperate condition [34]. No need to maintain the operational condition at high temperature can massively reduce the energy consumption, and facilitate the system installment and application to receive the wastewater for the treatment. Fourth, MFCs show the strong stability and higher resistance against the environmental variation, because of mixed microbial consortium such as *Shewanella* sp. and *Geobacter* sp. [35]. In summary, MFCs can make a breakthrough advance over the traditional anaerobic digester for the energy recovery.

However, the MFCs still have intrinsic bottlenecks for the energy generation and the cost effectiveness, due to the multiple energy losses during the MFC operation. First loss is the Coulombic loss, during the organic degradation and the electron releasing process from the anode [28]. It was reported that energy losses occurred in the MFCs, because some portions of wastewater organics were consumed by the microbial growth and the inefficient wastewater treatment [24]. Thus, the electrode potential and the electricity generation would be despaired, and it was estimated that the Coulombic loss could reach the level much more than 50%, even greater than the conventional anaerobic reactor in some cases [24]. Also, the capital cost would be the big issue for the MFC development. The electrode material and the cathode catalyst take up > 50% of the capital cost [30]. For example, it is reported that the platinum (Pt) is the commonly cathode catalyst for oxygen reduction reaction (ORR) in MFCs. However, the high cost is the challenge for the MFC manufacturing and the scale-up process. The economic limitation leads to difficult situation for MFC applications in the scale-up wastewater treatment process, relatively to the conventional wastewater treatment [26]. Thus, the improvement of the cathode material is in the process to find out the cost-effective cathode material. A variety of carbon-related materials are investigated, including graphite materials, carbon nanotubes (CNTs), and other nanocomposites [36]. Among these materials, graphene is promising as the material base to have more modification on the material surface with the additions of nanoparticles and other metal additives [37, 38]. The

introduction of graphene is possible to improve the electron transferring efficiency on the BES cathode, and promising to increase the energy generation and the wastewater treatment [39]. Moreover, another option using the biocathode inoculate with certain microorganisms (e.g., methanogens) can even generate organic such as methane and ethanol. For example, the biocathode acclimated with the *Methanobacterium palustre* could apply the electrons transferring in MFC to produce methane [40]. It was reported that the electro-methanogenesis at biocathode was the most leading and dominant process with good substrate conversion efficiency in MFC [41]. The success of the biocathode in MFC increase the possibility to add the algae into the cathode to have the synergistic relationship between electron transferring process and the microorganism, and the algal bioreactor is possible to be integrated as the important component to help MFC achieve more function, which will be introduced in the following sections.

1.3.2 Introduction to algae and algal bioreactor

With the increase of the population and energy crisis of the fossil fuel, an alternative fuel source is needed to solve the problem. Biofuels are promising to be alternative energy source for the traditional fossil fuels due to renewability, high biodegradability and environmental friendliness for the environmental protection [42]. There are multiple sources for the biofuel production, such as sucrose, lignin cellulose from wood [43]. However, these materials have low conversion rate and conversion processes are not economically feasible due to the large requirement of the special enzymes which is not profitable for the commercial production [44]. Thus, new biomass type is needed to solve the problems. Microalgae are promising alternatives with high potential to generate significant quantities of biomass and oil suitable for conversion to biodiesel. Compared with the traditional biofuel source (e.g., corn soybean), microalgae have much higher biofuel production rate, which can enable the decrease of the cultivation area of traditional biofuel production [45]. Based on the total biofuel production amount, the microalgae were reported with 91% oil productivity, playing significant role in the energy recovery (Fig. 1.4) [45]. Thus, development of the algal treatment is quite beneficial for the biofuel production in the future.

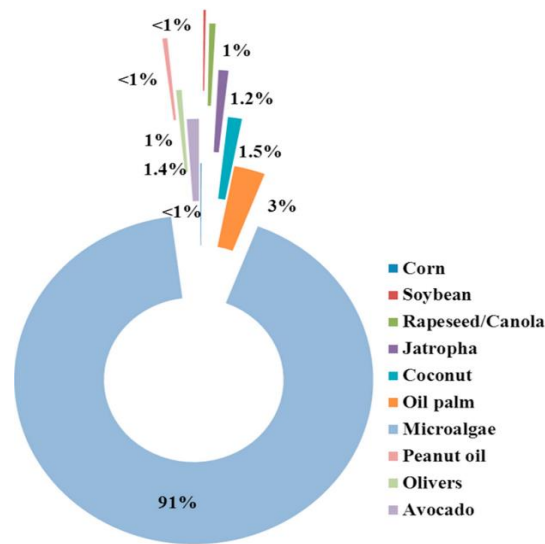


Figure 1.4 The rate of production of biofuel (L ha⁻¹) from microalgae including other feedstocks: The present scenario.

Reproduced with permission from reference [45].

The algae are able to uptake the carbon dioxide as the carbon source in the algae itself and serve the light illumination as the energy source for the cell survival and growth under the photosynthesis. One example of the microalgae, *C. vulgaris*, has the working mechanism to go through the photosynthesis and store the assimilated carbon to generate the biomass for the following treatment [46]. During the process, the N and P in the solution can also be absorbed into the cell to remove the nutrient in the solution. The general working mechanism is shown in the following figure (Fig. 1.5). Thus, the algae have the function for the carbon sequestration and efficiently obtain the nutrient to grow. Moreover, the microalgae have been estimated to have higher biomass productivity than the conventional plant crops in terms of land area required for cultivation, and have the potential to reduce GHG emissions through the replacement of fossil fuels [5, 9, 36]. It was reported that the high energy and biomass density for the microalgae enables the algal cultivation with only small area occupation to achieve the high productivity of biofuel. It was reported that microalgae had about 70% oil by weight of dry biomass, requiring only 0.1 m² year per kg biodiesel of land to produce 121,104 kg of biodiesel per year [42]. Thus, the algal cultivation and post-treatment strategy for the algae are promising for the biofuel production.

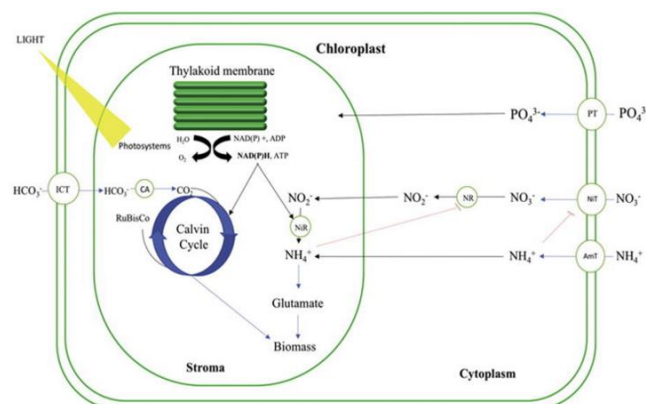


Figure 1.5 Working mechanism of the typical algae (*C.vulgaris*) under the photosynthesis. Reproduced with permission from reference [46].

The overall process to cultivate the algae and generate the biodiesel and bioethanol from the algae is shown in Fig. 1.6 [44]. These processes include cultivation and harvesting (upstream process), and the oil extraction and hydrolysis for the biodiesel production (downstream process). The upstream process can be combined with the wastewater treatment, using the algae to directly treat the wastewater to remove the organics and the nutrients [47]. Application of the algal treatment for the wastewater can improve the treatment efficiency, and also the organic and nutrient in the wastewater can be recovered by the algal uptake to generate more green energy. The synergistic relationship for the treatment strategy and the biofuel production can partially remediate the operational cost of the wastewater treatment. For example, it was reported that the benefit of the biofuel production was estimated at \$0.1 to 0.25 million per hectare of the high rate algal pond [47]. To further describe the application of the algal cultivation on wastewater treatment, this dissertation would be focused on the upstream process, with more discussion about how to improve the algal cultivation to obtain better algal growth and treatment efficiency.

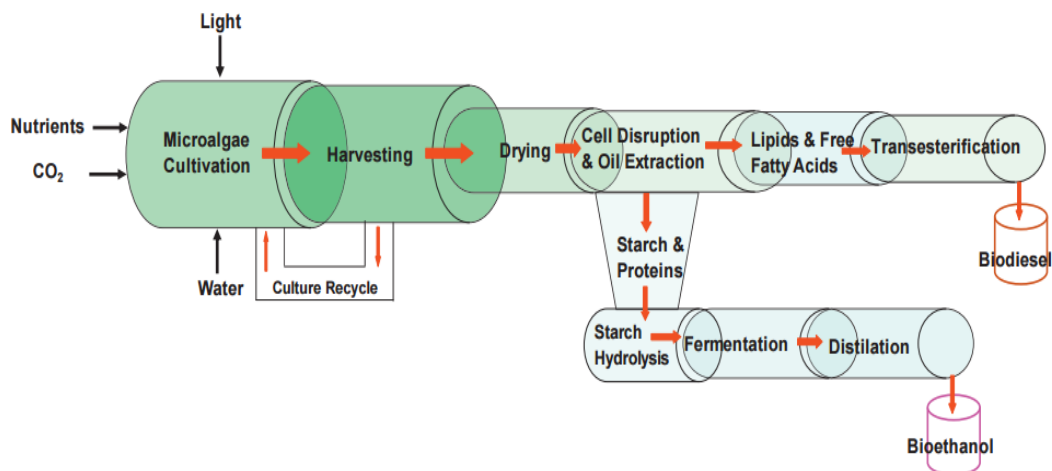


Figure 1.6 Biodiesel and Bioethanol production processes from microalgae.

Reproduced with permission from reference [44].

The configuration for the algal bioreactors needs extensive consideration for the sequestration of carbon footprint and bioenergy generation. The commonly-used algal bioreactors include open pond and various closed photo-bioreactors (PBR), with different shapes and characteristics. Open pond systems, such as open raceway ponds (Fig. 1.7), are most frequently used for the commercial cultivation, due to its easiness for the reactor construction and direct usage of the natural sunlight. However, the disadvantages including the difficulty of the condition control, direct exposure of the UV irradiation, contamination of the predators, and the easy interruption by the local climate would easily deteriorate the cultivation performance [48, 49]. In contrast, the closed PBRs can achieve high biomass productivities due to better control of culture variables [50]. Due to the construction of the closed system, the bioreactors would have less evaporation of CO₂ and the contamination can be avoided, which is beneficial for the algal growth condition. Also, the closed PBRs can have more versatile options for the reactor configuration or various orientations, including the horizontal, vertical and flat panel PBRs (Fig. 1.7), to facilitate the application of the specific reactors to satisfy the needs. These advantages of closed PBRs make the system to have stronger ability to obtain higher biomass productivity than the open system [50]. There was one study to construct the open and closed algal bioreactor to compare the algal productivity, and it was reported that the algal productivities in the vertical tubular photobioreactors and flat panel photobioreactors were 19.4 and 20.5 g m⁻² day⁻¹, respectively, much higher than those in the open raceway pond at 9.7 g m⁻² day⁻¹ [48]. However, due to more material requirement, the initial capital

cost of the closed system is much higher than those open system (open raceway ponds) [51]. The overall characteristic comparison between the open and closed system is summarized in Table 1, based on the previous reference [50].

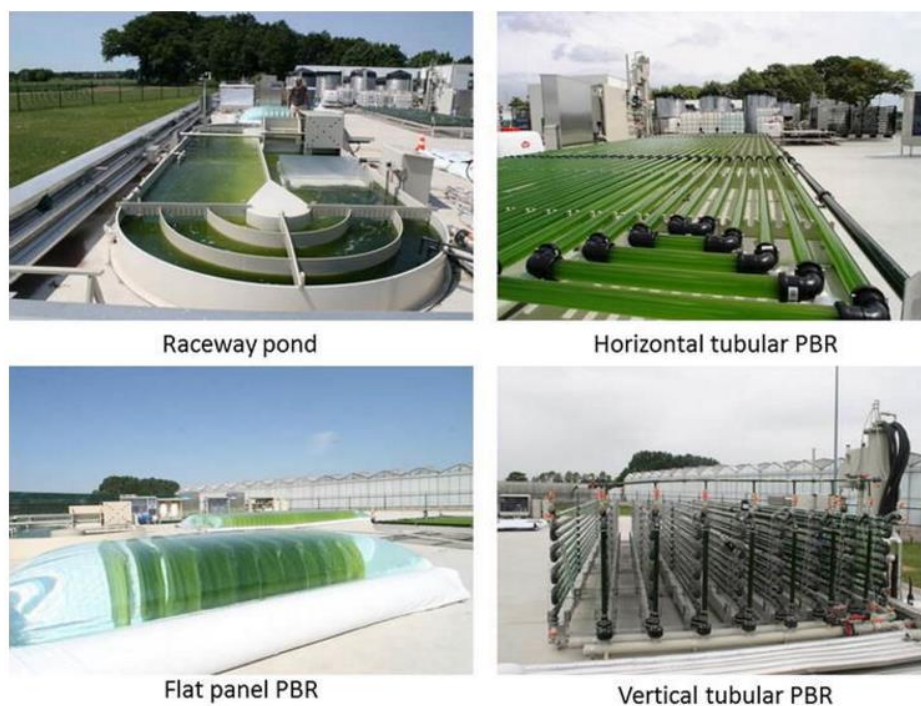


Figure 1.7 Photobioreactors of various configurations in Wageningen, Netherlands.

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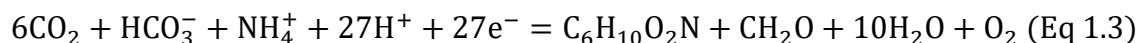
Table 1.1 Advantages and disadvantages of open and closed algal cultivation plants.

Reproduced with permission from reference [50]

Parameter	Open system (raceway ponds)	Closed systems (PBR)
Contamination risk	Extremely high	Low
Space required	High	Low
CO₂ loss	High	Almost none
Flexibility of production	Change of production between the possible varieties nearly impossible	Change of production without any problems.
Process control	Not given	Given

Weather dependence	Absolute, production impossible during rain	Insignificant, because closed configurations allow production also during bad weather
Biomass concentration during production	Low, approximately at 0.1-0.2 g L ⁻¹	High, approximately at 2-8 g L ⁻¹
Efficiency of treatment processes	Low	High

The strategy consideration to improve the performance of the algal reactor is significant, and the optimization of various operational factors to finally increase the algal growth rate is important for the energy conversion efficiency and biofuel productivity. The operational factors such as light illumination, temperature, solution pH and nutrients would significantly affect the growth rate. First, the light illumination is a significant factor for the conversion of chemical energy into the algal cells. Increasing the light illumination can maximize the algal growth until the light saturation point, and the photoinhibition would occur to inhibit the growth rate if the illumination extent exceeds the saturation point [47]. Thus, the illumination should be set at the optimal strength for the maximal growth rate. Second, for the temperature, the algal productivity would also increase with increasing pond temperature up to an optimum level, and excessive temperature would also inhibit the growth rate due to the high respiration and photorespiration rate to consume the organics to reduce overall productivity [50]. The photosynthesis and respiration processes need suitable temperature to guarantee the metabolism process and It was reported that the optimum temperature was at the range of 28 to 35 °C. Thus, the requirement for the optimal temperature on the algal growth leads to instability of the algal growth of the open system (Table 1). If the ambient temperature was below 20 °C, the algal growth would decrease to the low extent and the low temperature would even cause the irreversible damage on the algal growth [52]. Third, pH controlling also needs consideration for the high algal growth rate. The photosynthesis will uptake CO₂ from HCO₃⁻ or H₂CO₃ and hydrogen ions to convert them to the biomass, and generate oxygen, with the chemical equation shown in Eq. 1.3 [53]:



During this process, the solution pH increases after the photosynthesis during daytime, often reaching the level > 11. Under such high pH, the ammonia volatilization may be strengthened and

the ionic ammonium would be shifted to the free ammonia in the water. Though the conversion might benefit the N removal efficiency from the wastewater, the high concentration of the free ammonia will pose a huge toxicity for the algal growth, and the algal growth rate would be greatly inhibited [53]. Thus, the optimal pH is reported to be as slightly alkaline at ~8, and one typical example indicated that the optimal capture of CO₂ was under the pH at 7.8 for a marine *Chlorella* sp. for the highest growth rate [54]. For most algal species, the pH was maintained at a slightly alkaline pH of 7.8 and bicarbonate addition might be a good option due to its buffering ability and the supply of inorganic carbon [55]. Fourth, the nutrient levels would also affect the algal growth rate, and it was reported that the ratio of N:P of 16 N: P (7.3 g N: 1 g P) would be suitable for the algal growth due to the compatibility for the typical composition of algae (C₁₀₆H₁₈₁O₄₅N₁₆P) [56]. Thus, the suitable adjustment on the nutrient ratio of the feeding solution is another consideration for the growth improvement. The consideration of these factors would greatly benefit the algal growth rate, and ultimately the IPB performance with higher energy recovery.

Furthermore, the algal species is another key factor to affect the nutrient removal efficiency and the algal growth rate under various operational conditions. through biological interaction and processes. The selection consideration points for the algal species include the lipid productivity, tolerance against the extreme environment (e.g., temperature, highly contaminated condition) and the carbon fixation rate. The algae with high abilities on these points show the high application value to treat the wastewater and achieve high energy recovery. First, considering about the lipid productivity, *Chlorella* species can be selected as the competent algal species. It was reported that the *Chlorella* species can show the high growth rate, efficient removal of the nutrient and high rates of fatty acid accumulation in the wastewater treatment [57]. It was reported that the *Chlorella* species generally can produce high lipid at > 40 % of their own dry weight, with high potential for the biofuel production rate [45]. Specially, *C. vulgaris* and *Chlorella protothecoides* were found with the lipid productivity > 0.1 g L⁻¹ day⁻¹, much higher than other species [45], and *C. vulgaris* could even achieve the lipid productivity at 0.17 g L⁻¹ day⁻¹ [58]. Second, considering about the pollutant removal and tolerance against the extreme conditions of the wastewater (e.g., high content of heavy metals), *Chlorella* species has strong treatment capacity against diverse range of different pollutant compared to other microalgae [59]. For example, *C. vulgaris* showed strong

ability to remove the metals such as Zn(II) and Cd(II), in the wastewater [60]. Other several algae such as *Scenedesmus acutus*, *Chlamydomonas reinhardtii* and *Spirulina platensis* could also remediate other metals including Cr(VI), Hg (II), Cd(II), Pb(II) [60-62]. These species have relatively strong abilities to react against various wastewater conditions, and thus they are strong candidates to be applied in the treatment process to remove the organics and achieve high growth rate. Third, for the CO₂ fixation rate, it was reported that *Chlorella* species, especially *C.vulgaris*, showed much higher CO₂ fixation rate, at 80-260 g m⁻³ h⁻¹, than other species such as *S. platensis* and *Euglena gracilis* [63-65]. Thus, adoption of *Chlorella* species can offer significant benefits of direct capture of atmospheric CO₂, which can bring significantly economic value on the green sustainability. Therefore, the utilization of suitable algal species, such as *C.vulgaris*, can significantly enhance the CO₂ fixation, guarantee the wastewater treatment and even achieve the great growth rate. To summarize, the selection of the suitable algal species is influential on the biofuel production, wastewater treatment and other beneficial purposes.

1.3.3 Introduction to IPB system integrating BES and algal bioreactor

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As introduced before, MFCs use electricigens to degrade the organic in the wastewater to directly transfer the electrons to complete the electric circuits to generate the electricity [26, 66], and the direct biological conversion of organic chemical energy into electricity enables MFCs to obtain higher energy conversion efficiency (~44%) than that with anaerobic treatment (e.g., anaerobic digester) (~28%) [24, 67]. However, there are still some functional limitations to optimum performance of MFCs for energy recovery and nutrient remediation of wastewater. For example, electricity generation at the cathode requires oxygen as an electron acceptor, so aeration to supply oxygen is commonly needed. Aeration is a major energy consumer of domestic wastewater treatment [68-70], so eliminating aeration will greatly reduce energy consumption in MFCs and move this technology towards net positive energy recovery [28].

To address the need for oxygen supply, algae and/or photosynthetic bacteria may be incorporated into the MFCs to construct IPB system. Photoautotrophs (algae) can use photosynthesis to produce oxygen as the electron acceptor for electricity generation in the cathode [3, 4]. In addition to oxygen supply, photoautotrophs (algae) can also offer benefits including removal and potentially recovery of N and P macro-nutrients, removal of additional organic compounds and production of algal biomass that could be converted to biofuels or other value-added products [26, 57, 66, 71-75]. Algae naturally grow well in wastewater, and their nutrient removal services also provide excellent motivation to consider integration of algae into the MFCs [71-73]. The use of wastewater as a medium for algal growth for production of biomass for biofuels has been of interest for some time, with reduced costs of biofuel production utilizing a freely available source of freshwater and nutrients [57, 76-78]. Thus, the incorporation of the algal bioreactor into the MFC cathode to be the IPB system is an effective option to eliminate the aeration requirement and improve the nutrient removal efficiency, and the general integration concept is shown in Fig. 1.8 [79].

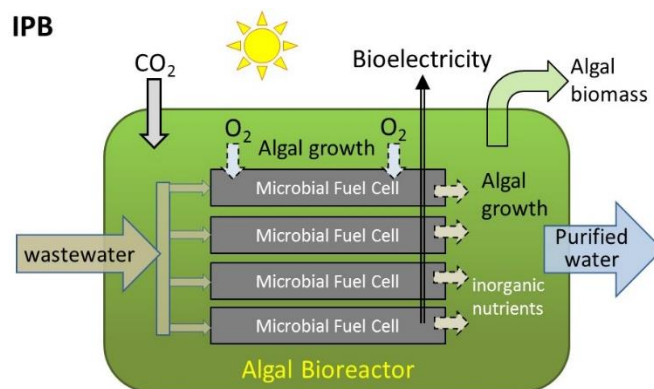


Figure 1.8 Basic schematic of IPB system showing how the MFC function can be supported by algal bioreactor.

Reproduced with permission from reference [79].

A chief benefit of the IPB system by incorporating the algae into MFC cathode is to improve the oxygen production to improve the ORR [80]. Algae attached to the cathode electrode evolve molecular oxygen from photosynthetic light reactions, and this oxygen diffuses to the catalytic sites on the cathode electrode [81, 82]. This is much more efficient than mechanical aeration of the cathode solution where dissolution of oxygen only happens on the surface of air bubbles, and thus oxygenation by algae is a greatly promising alternative to the mechanical aeration for greater

power output [3, 83, 84]. Suspended algae can also reduce the need for aeration. Li et al. constructed an IPB system with a tubular MFC anode installed inside an algal bioreactor, providing oxygen to the cathode (Fig. 1.9) [3]. Algal photosynthesis resulted in much higher oxygen concentrations during the light period ($\sim 20 \text{ mg L}^{-1}$) than with mechanical aeration ($\sim 8 \text{ mg L}^{-1}$) supplied during the dark period, resulting in higher electricity production in the IPB with the maximum power density at 2.2 W/m^3 [3].

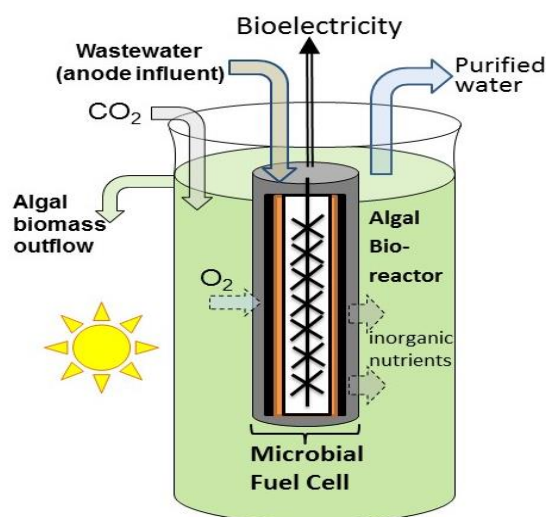


Figure 1.9 Schematic of IPB system using algae growing suspended in the catholyte solution and on cathode surfaces, assisting the cathode with oxygen supply and take up nutrients.

Reproduced with permission from reference [3].

Algae can serve as a buffering agent in the cathode medium (catholyte). Reduction of oxygen at the cathode surface results in increased alkalinity of the algal bioreactor catholyte solution [80]. Algal photosynthetic inorganic carbon demand withdraws CO_2 from the medium resulting in a drift to higher pH as the carbonate equilibrium compensates for the CO_2 uptake. In the cathode, photosynthetic organisms can also buffer the pH increase due to proton production by respiration at night [3], and additional CO_2 often supplied to optimize algal growth can also reduce pH [72, 85]. The pH increase and decrease during the daytime and night serve as buffering agent to maintain cathode pH. Another bonus of a pH increase during the light period is for harvesting algal cells in the bioreactor chamber as higher pH increases cell aggregation and sedimentation [86].

For the nutrient removal, introduction of algae in the cathode can also promote nutrient (e.g., N, P) transformation and removal from wastewater, which might not be possible solely by MFCs [72]. Cells cultivated in the cathode chamber receive the effluent from MFC anaerobic anode, mostly containing ammonium, and phosphate. The cells can take up and incorporate these nutrients into biomass to simultaneously remove nutrients, and generate biomass which could be used for biofuels production. In general, electricigens in the anode can effectively remove organics under anaerobic conditions, removing up to 90% of chemical oxygen demand (COD) related to load of organic compounds, but some remaining organic compounds may transfer to the cathode [87, 88]. Some algae have the capacity to grow mixotrophically and could help with additional organic carbon breakdown. More importantly, N and P removed by photoautotrophic cells in the cathode help address limited capacity of MFCs for the nutrient removal [89]. In a lab-bench IPB, high nutrient removal efficiency can be achieved (removal of >90% removal of ammonium-N, 82% P) along with algal biomass production (120 mg L^{-1}) [3, 90]. Algal cell density in the cathode chamber will also affect the nutrient removal efficiency; a higher concentration generally contributes to higher removal efficiency [91]; however, negative effects of high biomass density can also occur, including reduced growth rate related to light penetration of dense cultures, maintenance of sufficient inorganic carbon supply and greater pH fluctuation.

To improve the IPB performance, the selection of the suitable algal species and bacteria is important. Particularly, identified use of algae in IPB is dominated by green algae and predominantly *Chlorella* (*Chlorophyta*), a unicellular unflagellated green algal genus which can grow in colonies, reaching high biomass densities. *Chlorella* is also a model alga for laboratory studies for many decades [92], more recently attracting interest for cultivation for food, nutritional supplements and biofuel production [93]. The most commonly used species is *C. vulgaris* which is tolerant to wastewater and has typically been employed as the algae used in the biocathode to produce oxygen as electron acceptor in IPB [83, 91, 94-105]. *C. pyrenoidosa* is another common research organism which has been used in BES [106]. *Chlorella* species growing in high nutrient and organic wastewaters show high growth rates, effective removal of nutrients, high rates of fatty acid methyl ester production as precursors to biodiesel [57, 74, 107-109]. However, as the functional aspects of these integrated systems become characterized in more detail, there may be

unexplored taxa which offer functional advantages to IPB systems. Thus, more studies are needed to study the performance of other species in the IPB system, and possibly the more suitable algal species can be found to be inoculated into the IPB cathode for the better performance in the wastewater treatment and energy recovery.

In the conventional IPB system with the algae in the cathode, the treatment of the real wastewater will also introduce the bacteria into the cathode. Thus, there are multiple species of algae and bacteria detected in the IPB cathode due to various wastewater feeding [4]. The understanding of the interaction of algae and bacteria in the cathode would also help to know the directions to improve the IPB performance. In an IPB, CO₂ supply from bacterial respiration can directly diffuse to photosynthetic cells for the algal uptake, while the O₂ production from the algal photosynthesis can benefit the bacterial respiration [79]. Thus, the mutually beneficial interactions can boost the growth and species richness of algal-bacterial community. Then, high species richness in the algal-bacterial communities improves the stability and reliability, and the strong community can finally improve the treatment ability of the IPB for versatile types of wastewater [110-112]. However, currently, there are still limited information about the interactions among specific algal species and bacterial species, since there are few IPB studies directly about their interactions during the process of electricity generation. Thus, more extensive studies about these algal-bacterial interactions in the IPB cathode would be a significant target to directly improve the cathodic microbial community function to more effectively treat various types of wastewater and generate higher energy in the future. This dissertation would give more detailed discussion about the development of IPB system.

1.4 Introduction to mathematical modeling for BES

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The performance of various BES is affected by biological, physical-chemical and electrochemical aspects (e.g., microbial community, configurations, electrode material, etc.) [113-115]. Considerable studies have been conducted to optimize the above aspects [26, 28, 116-121]. However, the BES development for energy recovery from wastewater treatment still has

bottlenecks [114, 122]. For example, BES may not replace anaerobic digesters to treat the high-strength wastewater [123], and the scale-up BES cannot achieve a similar performance in current generation as that of the lab scale systems [114, 124, 125]. How to improve the BES performance is still challenging, and thorough understanding of these significant aspects which limit the overall BES performance is needed for future improvement.

A variety of works have been conducted to study the effect of design and operational parameters on BES performance, such as reactor configurations and scales, electrode materials, electrode surface areas and the types of electron donors [28, 88, 126-133]. However, because of the lack of the sensing technology, some aspects are hard to be measured *in situ*, such as microbial community composition, biofilm thickness and its growth rate, redox mediator transferring, and substrate utilization rate of bacteria attached on the electrode [134-140]. These hard-to-measure aspects are likely to be key components for understanding the mechanisms of electricity generation and improving the efficiency of BES.

Mathematical modeling can be a powerful platform to investigate the effect of above aspects on the overall BES performance [115, 120, 141-143]. The use of mathematical models may provide analytical description of these hard-to-measure aspects, and thus reduce the measurement effort. The versatility of mathematical models enables the conversion of complexly systematic phenomena into a relatively simple series of mathematical expressions to describe the effect of each component on the overall output [115, 144]. In general, mathematical modeling is developed and performed in two approaches. One approach is to derive model based on engineering/physical laws governing the system processes, and this is called engineering modeling [145, 146]. Most engineering models are deterministic models, because the output of the model is determined by the parameter values and the initial conditions [147]. For example, differential equations (DEs) are used as main mathematical equations in the engineering models due to their strong ability to embody the dynamics of BES [115, 148-151]. The parameter values and initial conditions are set depending on specific situations to operate the model. The main equations are combined with physical, chemical and electrochemical principles to solve specific problems. For example, Monod-type equations are created for describing the growth curve of specific microorganisms in

a certain living environment [136, 152, 153]. Nernst–Michaelis–Menten equations are applied to calculate the amount of Nernstian electron transferred to embody bio-anode kinetics [154-156], and Nernst-Planck equations are applied to represent the ion diffusion through the interface or membrane of a system [157-160]. The other approach is statistical modeling, which is developed based on the data collection from BES [145, 146], and is also valuable for characterizing the system input-output relationships, especially when there is limited engineering-domain knowledge to characterize the complex mechanisms of electricity generation and organic material removal. In addition, the statistical models can better capture the system uncertainty and remedy the error originated from engineering models for better system quantification [145]. A model methodological tree is summarized to make classifications for all types of BES model in the following Fig. 1.10 [161].

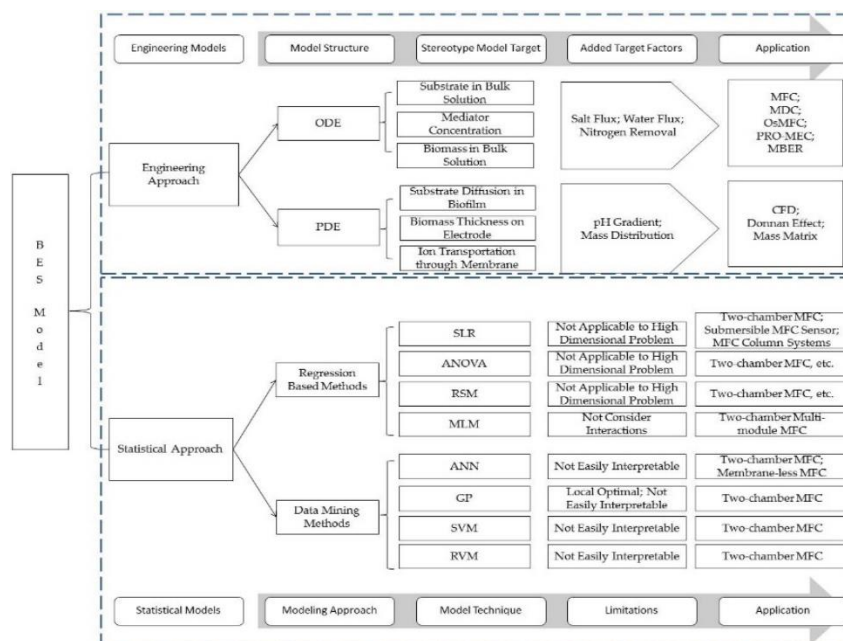


Figure 1.10 BES modeling methodological tree.

(ODE: ordinary differential equations; PDE: partial differential equation; MFC: microbial fuel cell; MDC: microbial desalination cell; OsMFC: osmotic microbial fuel cells; PRO-MEC: pressure-retarded osmosis/microbial electrolysis cell; MBER: membrane bioelectrochemical reactors; CFD: computational fluid dynamics; SLR: simple linear regression; ANOVA: analysis of variance; RSM: response surface methodology; MLM: Multi-Lasso Model; ANN: artificial neural network; GP: genetic programming; SVM: support vector machine; RVM: relevant vector machine).

Reproduced with permission from reference [161].

Conventionally, for the MFC modeling, ordinary differential equations (ODE) is commonly applied to simulate the system performance on the organic degradation and the electricity generation. The advantage of ODE is to allow the fast computation and provides advantageous platform for real-time process control and optimization of BES [162, 163]. Some ODE stereotypes can successfully model the dynamics of substrate concentrations and various microbial populations in BES, and these ODE stereotypes are also the foundations for the future ODE development [135, 150, 164].

ODE stereotypes are constructed based on the principle of mass balance to illustrate the dynamic response of substrate concentration and biomass in the bulk solution in anode chamber [135]. Several parameters were considered in the ODE: the rate of substrate consumption, the rate of growth and death of biomass in the bulk solution, and the rate for the electrochemical oxidation of reduced mediator at the anode surface. These considerations have effectively tracked the source and sink for the dynamic change of substrate and biomass in the bulk solution at each moment in time, and a generic form of ODEs was constructed [135]. However, the ODE cannot predict the dynamic change of microbial biomass on the biofilm. The equations simplified the microbial community into overall biomass, which cannot specify and distinguish the contribution of each type of functional microbial species (e.g., electricigenic and acetoclastic methanogenic microorganisms) in the anode chamber [135].

More general ODE stereotypes were developed with more focus on the competition and contribution among various types of microorganisms in the anode chamber of BES, including two types of BES (i.e., MFC and MEC) [150, 165]. The previous works successfully integrated multiple kinetic equations such as Monod equations to take many parameters into account, including substrate consumption rate, biomass growth rate, and even the impact of mediator on the dynamic simulation (Fig. 1.11). The related ODE stereotype equations are listed and explained in the Table A1 in Appendix A.

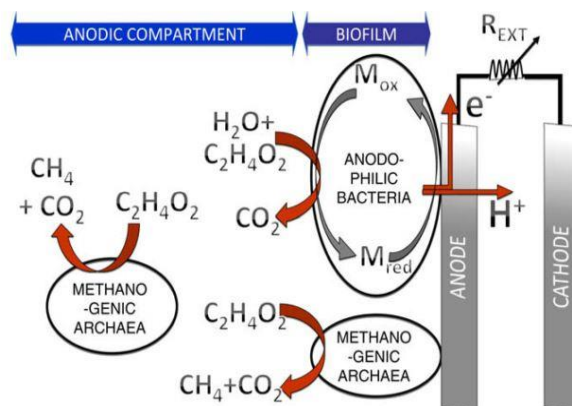


Figure 1.11 Conceptual MFC model showing carbon source conversion and biofilm activity.

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To satisfy the increasing needs for mathematical models to achieve better simulation and prediction of the BES performance for wastewater treatment and energy recovery, more mathematical models are developed for other systems, such as MDC [166, 167]. For the example of the MDC simulation, the upgraded model can predict the salt concentration in the desalination, anode and cathode chambers, and be used to study the impact of different parameters on the system performance to optimize the salt removal efficiency. The model can even predict the dynamic response of the system output by changing the influent concentration of acetate, the external resistance, and the influent salt concentration. The developed ODEs can accurately simulate the quantitative influence of different parameters on MDC performance. The best acetate flow rate, influent salt concentration, and salt solution flow rate were found according to the model output [166], and thus the developed ODEs can be applied as a valuable tool for future MDC development and optimization. The configuration and the example simulation results are shown in Fig. 1.12 [166].

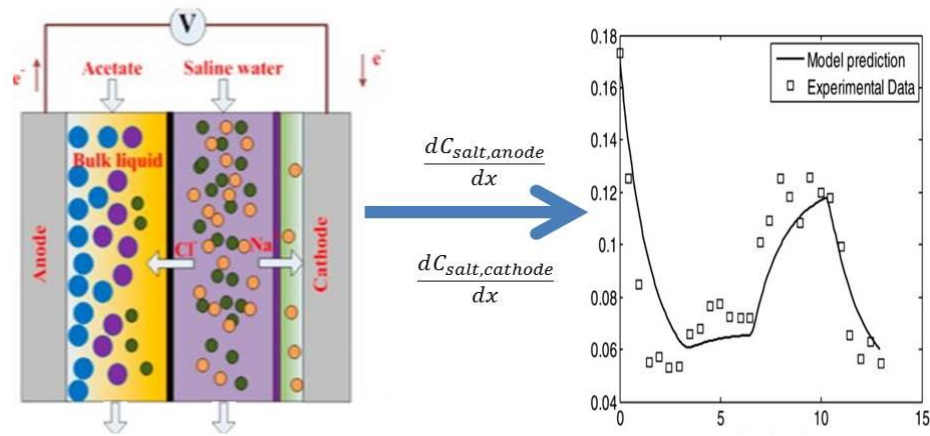


Figure 1.12 System configuration and model output of an MDC.

Reproduced with permission from reference [166].

Similar to the above strategy to update the models to enable the simulations for more systems, it is also possible to develop the ODE model with more component additions to simulate the IPB performance and give the optimization suggestion for the performance improvement. The component additions include the impact of the oxygen concentration on the MFC cathode, the dynamic change of the N and P concentration in the cathode, and the impact of the illumination on the IPB performance during daytime and night. More details are shown and discussed in Chapter 8.

Though the convention ODE models have many strengths to be applied for the BES modeling, the parameter adjustment for the model construction is required. However, due to too many parameters needing the adjustment and determination for the modeling construction, the laboring consumption to construct the model would be massive, especially for the complicated models with multiple parameters to adjust [145, 146]. Thus, another tool is needed to help the parameter adjustment and guarantee the model accuracy. The statistical model based on Bayesian methods can be introduced to solve the problem [168]. With the integration of the traditional engineering models and statistical models, the advantages of both models can be integrated together to improve the model accuracy and reduce the laboring consumption on the model construction. More details will be described and discussed in Chapters 6 and 7.

1.5 Introduction to application of stable isotope probing for MFCs

The analysis for the microbial communities in MFCs is significant to know more information about the interactions of various microbial communities and thus know more information about which species are responsible for the electricity generation or significant for the wastewater treatment. The information of the community composition or relative activity can give valuable information about what microorganisms are responsible for the good MFC performance. It was reported that dominant abundance of exoelectrogenic microorganisms in the anode often were often accompanied with the good electricity generation [169, 170]. Thus, this result suggests that the dynamic change of the exoelectrogenic activities in the microbial community will finally affect the MFC performance. Accurate and detailed investigation on the dynamic performance of the microbial community is significant to give a picture on the relationship between the community condition and the MFC performance (e.g., current generation and wastewater treatment), and this information can serve as a guide to improve the MFC performance.

Suitable methods are needed to accurately analyze the microbial community in MFC to obtain more information of microbial communities in BES [171]. A variety of traditional techniques have been used before to study the microbial community, such as quantitative polymerase chain reaction (qPCR), denaturing gradient gel electrophoresis (DGGE), fluorescence *in situ* hybridization (FISH), and pyrosequencing [171-173]. These methods could derive the existence of certain species living in the MFCs, to tell the community composition and relative abundance of various species. However, these traditional methods are hard to give the exact explanation or evidence directly related to the electrochemical activities of these species in MFCs. Sometimes, the high abundance of key microorganisms is not always related to the good MFC performance [171]. Sometimes, specific organisms with low abundance could even have higher proportion contribution of the total current generation in MFC, such as *Shewanella putrefaciens* PS-1 and PS-2 [174]. Thus, another method is needed to directly detect the strength of the gene expression or the function of the community in the MFCs.

Stable isotope probing (SIP) is one emerging *in situ* technique powerful for the identification of active microorganisms that carry out specific metabolic processes within complex environments [175]. The working mechanism is to identify the active microorganisms by the selective recovery and analysis of isotope-enriched cellular components, to obtain the information about how

actively the microorganisms function in the isotope-related pathways to collect the isotope. In MFCs, majority of SIP studies used and quantified the ^{13}C -labeled substrate in the cellular biomarkers (e.g., DNA or RNA), to determine what metabolic process the microorganism goes through. For example, SIP could be applied to determine what species were dominant in MFC with various organic supply. It was reported that with acetate as the organic supply, the dominant phylotypes for the carbon uptake include *Geobacter* and *Rhodocyclaceae*, while both *Enterobacteriaceae* and *Geobacter* were dominant under the glucose supply in MFC [176]. The abundant options of the ^{13}C -labeled substrate targeted for the function of specific microorganisms in the MFCs enable SIP technique to be universally available for obtaining the detailed metabolic information related to the current generation in MFCs. Thus, SIP is a competent technique to directly tell the metabolic metabolism of the specific microorganism in the process of the organic conversion to produce electricity in MFC. More detailed examples are described in Chapters 4 and 5, for the species *Shewanella Oneidensis* MR-1 in the process of lactate and formate degradation in MFC.

1.6 Outline

This dissertation is composed of eleven chapters. The overall goal is to develop the IPB systems to achieve better energy recovery, higher treatment efficiency, system optimization and high application value towards sustainable wastewater treatment. More specifically, the dissertation summarizes and solves the following objectives.

The first objective is to improve the MFC performance by using the alternative cathode material to reduce the capital cost of MFC, and finally increase the possibility to apply the MFC in the real wastewater treatment with low capital and operational cost. Nickel (Ni) is reported to be with higher electro-catalytic ability in MFC and MEC due to its high conductivity to be suitable as current collector [177]. It was even reported that addition of Ni sheet onto the stainless steel could even replace the traditional Pt as the cathodic catalyst for ORR, with comparable current generation [178]. Thus, the study about the performance of using Ni as cathode material has high potential to increase the cost effectiveness of the MFC construction, and this improvement will finally benefit the IPB system by suggesting a competent cathode material to apply for greater energy generation.

The second objective is to investigate the various operational conditions on the BES performance in the energy recovery efficiency. One important BES type is MEC, which needs the external power supply to overcome the potential gap to process the hydrogen evolution reaction (HER) to generate the hydrogen gas (H_2) in the cathode component [179]. Also, due to the anaerobic condition in the MEC anode acclimated with various electricigens and methanogens, methane (CH_4) generation is frequently occurring in the process of the carbon conversion when treating the high-strength wastewater with high organic loading rate [180]. Thus, the simultaneous generation of H_2 and CH_4 are often occurring when using MEC to treat the high-strength wastewater. It is good news that H_2 and CH_4 are both gas with high energy content, which has high potential to be treated in next step to release huge energy for the benefit of people [181]. Biohythane, which is composed of H_2 and CH_4 , is a new concept of energy gas, as a promising alternative to the traditional fossil fuel due to its high fuel combustion efficiency [182, 183]. Investigation of how to optimize the operational system can benefit MEC function for higher production rate of biohythane, to obtain higher energy generation efficiency [180]. This study can reflect the interaction between the operational condition and the system output, finally giving some suggestions for the optimization of the operational condition of IPB system.

The third objective is to apply the technique of stable isotope probing by using ^{13}C to study the metabolic process of modeling electricigen, *Shewanella oneidensis* MR-1, in the organic degradation process in MFC. The understanding of the metabolic process related to the current generation in MFC can give valuable information about how to adjust the ratio of various organic supply to facilitate the organic degradation and electricity generation in MFC. The information obtained in this objective can facilitate the interpretation of the fundamental mechanisms of the electricigens in the process of electron transferring.

The fourth objective is to optimize the mathematical model by the integration of engineering-based and statistics-based models together to obtain the advantages of both models to obtain the integrated ensemble model to improve the model function to efficiently capture the dynamic change of the MFC performance and give accurate prediction on the MFC performance [184]. Furthermore, the new model strategy can increase the efficiency to construct the MFC model [185-187]. This improvement on the model function can be further extended to the simulation of the IPB performance. The extensive development of traditional MFC model to cover the IPB

performance can help optimize the IPB performance for greater energy recovery and wastewater treatment.

The fifth objective is to improve the algal harvesting efficiency from the IPB system. Since the algae is the dominant proportion of the energy recovery from the system, the improvement of the algal harvesting strategy can benefit the ultimate energy generation efficiency. Since the algal growth in the attached mode on the specific substrate tends to higher growth rate [188], the algal biofilm cultivating system is promising to improve the IPB performance in the angle of the algal harvesting amount and the increase of the energy recovery.

The sixth objective is to scale up the IPB system to treat the real wastewater, and evaluate the application value of the IPB system in the long-term operation under seasonal variance change. Compared to the highly costly enclosed photobioreactors, the open pond system can be considered as the simplest and most economical method for the algae cultivation [188]. Thus, the open pond IPB system with the horizontally placement of MFCs are applied, fed with the real wastewater from Virginia Tech. The fulfillment of this objective can indicate if the feasibility and capability of the scale-up IPB system can satisfy the public requirement on the wastewater treatment and energy recovery in the current stage.

Chapter 2 Ni-Coated Carbon Fiber as an Alternative Cathode Electrode Material to Improve Cost Efficiency of Microbial Fuel Cells

(This section has been published as: Shuai Luo and Zhen He. "Ni-coated carbon fiber as an alternative cathode electrode material to improve cost efficiency of microbial fuel cells." Electrochimica Acta 222 (2016): 338-346.)

2.1 Introduction

Microbial fuel cells (MFCs) have attracted a great attention as an emerging technology for sustainable waste treatment and bioenergy recovery. In an MFC, electrochemically-active organisms oxidize organic compounds and release electrons to an anode electrode, which transfers the electrons to a cathode electrode for reducing terminal electron acceptors. When treating wastewater, MFCs can generate electrical energy directly, require low energy input, and produce a small amount of biosolids that need further disposal [32, 66]. In the past two decades, MFCs have been greatly advanced in terms of understanding microbial-electrode interaction, new catalyst/electrode materials, reactor design and operation, and electrochemistry [26, 189]. One of the major challenges for MFC development is system scaling up. There have been efforts to enlarge MFCs to a scale of several hundred liters for treating actual wastewater [190]. Despite a preliminary analysis in a recent study that the capital cost of MFC systems could be lower than some small-scale wastewater treatment facilities [191], it is generally acknowledged that MFCs systems are expensive [192, 193]; thus it is of great interest to explore cost-effective materials for MFC development.

Cost efficiency of an MFC can be improved by using low-cost materials that do not significantly sacrifice the performance. As an electrochemical system, electrode materials play an important role in MFC performance and cost. An anode electrode material can affect the power generation of MFCs through influencing microbial growth and electron transfer from microorganisms. Thus, the appropriate anode electrode material should have a large surface area for microbial attachment, good biocompatibility, and low resistance. Popular materials used as anode electrodes include carbon brush, carbon cloth, and granular carbon/graphite [194, 195]. A cathode electrode can also greatly affect power generation, due to high reduction overpotential caused by slow rate of oxygen reduction potential (ORR) on the carbon surface [196, 197]. For example, the mainly limiting

factor for current generation occurs in the cathode, because the kinetics of ORR in cathode is lower than the organic oxidation in the anode, causing larger overpotential of cathode than anode [198, 199]. The slow kinetics to limit MFC performance in ORR is possible because of the requirement of high activation energy (498 kJ mol^{-1}) to cleave the O=O bond of oxygen molecule [198].

The research of MFC cathode electrodes heavily focuses on catalysts, because of abovementioned ORR kinetics [200]. The function of a catalyst is to reduce the activation loss for stronger ORR, and the conventional catalyst such as platinum (Pt) can dramatically improve ORR [196]. However, the Pt-based cathode accounts for almost 50% total cost of lab-scale MFC systems [201]. Alternative catalysts are of great interest, and among them, activated carbon (AC) has been demonstrated effective in ORR catalysis with a much lower cost [202, 203]. On the other hand, the cathode electrode, which is also the supporting material for catalysts, has not been studied in details like that of catalysts. Carbonaceous materials (e.g., carbon paper, graphite granules) are the most widely utilized cathode electrode materials in MFCs, owing to their strong mechanical strength and high porosity to scaffold a large amount of catalysts for better ORR performance [204]. Among those materials, carbon cloth (CC) is commonly used as cathode electrodes [177], and the CC coated or pressed with AC (AC/CC) has been proved to have long-term stability and outstanding electrochemical performance to treat the real wastewater [32, 191, 194]. However, alternative cathode electrode materials that can produce similar performance to CC but be cheaper will still be worth exploration.

In this study, a commercially available and low-cost carbon fiber (CF) coated with nickel (Ni) was investigated as an alternative cathode electrode material to support AC catalysts. Cost efficiency, which is rarely reported in the previous studied of electrode materials, has been analyzed for the tested electrode materials. Ni has been studied as an effective electrode material [202], and Ni foam could act as a current collector for AC catalysts to achieve comparable performance to the MFC using Pt-based cathode [177]. The objectives of this study were to demonstrate that this nickel-coated carbon fiber (Ni-CF) could outcompete CC as a cathode electrode for MFCs in terms of performance and cost, and to examine the effects of AC loading on the cost efficiency. For comparison, CC and Ni-CF coated with AC catalysts (AC/CC and AC/Ni-CF), CF without Ni (CF), and Ni-CF coated with acidic/alkaline washed AC (W-AC/Ni-CF) have also been studied for

power generation and cost efficiency. Cost efficiency was quantified using numerical standard (mW \$⁻¹) [200].

2.2 Experimental

2.2.1 Cathode material preparation

The commercially available CF and Ni-CF (Toho Tenax, Rockwood, TN, US), and CC (PANEX 30PW03, Zoltek Corporation, St. Louis, MO, US) were used as the cathode materials (Figure B1). It should be noted that CC (flat sheet) and CF (thread type) are very different in terms of dimensions, and the criterion to determine the amount of CC or CF is based on full coverage of the cation exchange membrane by one layer of the cathode material. All electrode materials were pretreated by acetone [205], and then coated with AC powder if needed. To coat AC on the electrode, Ni-CF or CC was submerged in a solution containing 100 mL ethanol, 4 g AC (specific electrode coating amount at 23.53 mg cm⁻²) and 2.5 mL 60 wt% PTFE (actual PTFE amount at 2.08 g) in an air-tight container (150 mL) to make the AC/PTFE mass ratio as 1.92, following the same procedure of previous work [206]. Afterwards, the AC-coated Ni-CF (AC/Ni-CF) and CC (AC/CC) were dried and heated at 370 °C for 30 min, and were ready for tests. The method to make acid/alkaline washed AC/Ni-CF followed a previous study [207], and the temperature of heat treatment for AC/Ni-CF after alkaline washing rose up to 500 °C in a muffle oven [208]. The unit cost of each cathode material is listed in Table 2.1. Cost efficiency was calculated by using maximum power derived from polarization tests and normalized to the total cost of only the cathode material (cost efficiency = $\frac{\text{maximum power}}{\text{total cost of cathode material}}$) [200]. Both electrochemical test and MFC test were conducted to compare the electrochemical performance among various electrode materials and test the effect of AC loading on cathode electrode. In the comparison of electrochemical performance of basic electrode material, electrode materials coated without and with AC catalyst were tested. In the test of impact of different initial AC coating amount on the electrode performance, different AC amount was coated on Ni-CF with selected initial coating amount of AC powder (0, 2, 4, 6, 10 g) and corresponding volume of 60 wt% PTFE binder (actual PTFE amount at 0, 1.04, 2.08, 3.12, 5.20 g), to make the AC/PTFE mass ratio constant as 1.92 (Table 2.2). Thus, the supposed AC coating densities on the electrode were 0, 11.76, 23.53, 35.29 and 58.82 mg cm⁻², respectively, and the actual AC loading densities (mg cm⁻²) in different

experimental groups were indicated in the following results and discussion, calculated by multiplying 0.66 on total weight difference before and after AC coating process to exclude PTFE loading (derived from mass ratio between AC and total weight ($1.92/(1+1.92)$)). All experimental design for the real tubular MFC test (section 2.2) is listed in Table 2.2.

Table 2.1 Unit cost of cathode material.

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Cathode material	Unit cost	Additional material used
CC	\$ 45 m ⁻²	/
CF	\$ 0.027 m ⁻¹	/
Ni-CF	\$ 0.141 m ⁻¹	/
AC (4 g) /Ni-CF	\$ 1.445 m ⁻¹	100 mL ethanol, 2.5 mL 60 wt % PTFE
W-AC (4 g) /Ni-CF	\$ 7.722 m ⁻¹	100 mL ethanol, 2.5 mL 60 wt % PTFE, 50 mL concentrated H ₂ SO ₄ solution, 50 mL 3M KOH solution, and 4 g KMnO ₄ solid

Table 2.2 Experimental design for tubular MFC operation in this study.

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Experimental Group # and Experimental Target	Electrode Material	Amount of AC (g) (60 wt % PTFE (mL), actual PTFE amount (g)) needed to coat AC on the cathode electrode
#1 intrinsic base material	AC/CC	4 (2.5, 2.08)
	AC/Ni-CF	4 (2.5, 2.08)
	W-AC/Ni-CF	4 (2.5, 2.08)
#2 different coated amount of AC/Ni-CF	AC/Ni-CF	0 (0, 0)
	AC/Ni-CF	2 (1.25, 1.04)
	AC/Ni-CF	4 (2.5, 2.08)
	AC/Ni-CF	6 (3.75, 3.12)
	AC/Ni-CF	10 (6.25, 5.20)

Note: mass ratio of AC/PTFE is constant as 1.92 for all experiments; AC/CC represents activated carbon coated on carbon cloth; AC/Ni-CF represents activated carbon coated on Ni-coated carbon fiber; W-AC/Ni-CF represents acid/alkaline washed activated carbon coated on Ni-coated carbon fiber.

2.2.2 MFC setup and operation

Multiple tubular MFCs were constructed and placed horizontally in sequence from top to down with all the anodes electrode connected together while the cathode electrodes separated, and an external resistor of $10\ \Omega$ was connected in each anode-cathode circuit (Figure 2.1). In each MFC, the total anode volume was 280 mL (working volume: 200 mL; diameter: 4.7 cm; length: 15 cm). The anode electrode material was the pretreated carbon brush, and the cathode material was described as section 2.1 mentioned (Table 2.1). The anode and cathode were separated by cation exchange membrane (CEM) that had a surface area of $170\ \text{cm}^2$. The pictures of individual tubular MFCs are shown in Figure B2. The anolyte was synthetic wastewater, containing (per L of DI water): sodium acetate, 0.2 g; NH_4Cl , 0.15 g; NaCl , 0.5 g; MgSO_4 , 0.015 g; CaCl_2 , 0.02 g; NaHCO_3 , 0.1 g; KH_2PO_4 , 0.53 g; K_2HPO_4 , 1.07 g; and trace element, 1 mL [210]. The catholyte was 20 mM phosphate buffered saline (PBS, with the initial pH of ~ 7.1). The anodes were inoculated with anaerobic sludge from a local wastewater treatment plant (Radford, VA, USA), and cultivated for about one month until all individual MFCs achieved steady performance under the fixed resistance at $10\ \Omega$. Both the anolyte circulation and catholyte dipping rate were controlled at $30\ \text{mL min}^{-1}$ by peristaltic pumps without any refreshment during one operation cycle (BT600-2J, Langer Instruments Corp., NJ, USA). The MFCs were operated in a batch mode, and all the anolyte and the catholyte were completely refreshed every 24 hours at the end of each operation cycle. The reason to connect all MFC anodes together (Figure 2.1), was to keep the anode potential the same for all individual MFCs to maximally eliminate the anodic impact on MFC performance. Also, catholyte was shared for all MFCs to eliminate the interference of catholyte pH and conductivity on the performance test of all MFCs. Thus, the MFC setup could guarantee that the performance difference was only attributed to the cathode material difference. Exchange of cathode material on different individual MFCs would also be done to eliminate the interfering impact of CEM difference on MFC performance, mentioned in section 3.2, and the method was to strip off the cathode material from original MFC and assemble them onto the CEM of other tubular MFCs.

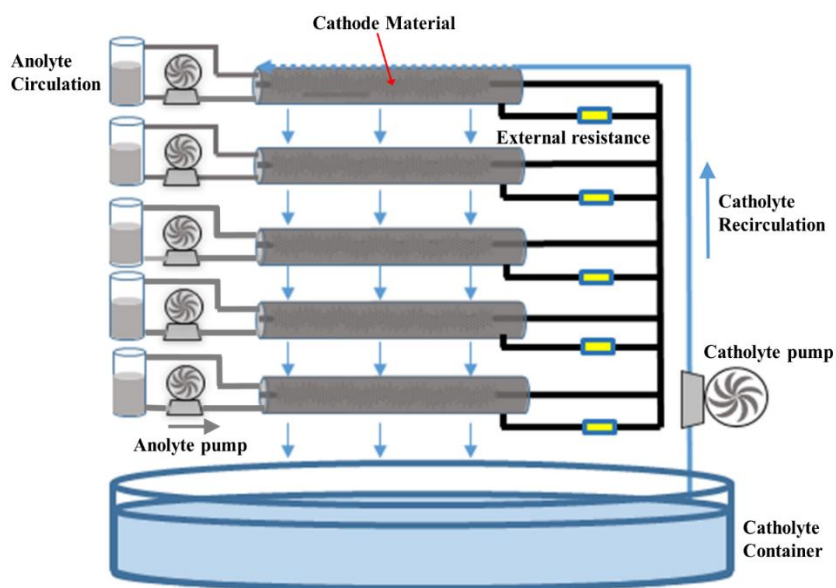


Figure 2.1 Setup schematic of five tubular MFCs in this study (five tubular MFCs with anode connected together, but cathode material separated with each other)

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2.2.3 Measurements and analyses

The cathode electrodes were characterized in both three-electrode systems and MFCs. To evaluate the electrochemical properties of the materials using linear sweep voltammetry (LSV) and electrochemical impedance spectroscopy (EIS), a three-electrode electrochemical cell was set up containing a working electrode (the cathode material), a reference electrode (Ag/AgCl, 0.201 V vs standard hydrogen electrode, SHE), and a counter electrode (Pt wire). LSV was performed in 50 mM PBS with a potential range from 0.4 to -0.8 V (vs reference Ag/AgCl electrode; scan rate: 5 mV s^{-1}), and EIS was conducted at scanning frequency of 1×10^5 to 2×10^{-3} Hz by using a potentiostat (Reference 600, Gamry Instruments, Warminster, PA, USA).

In the MFC test, the weight of each cathode material (e.g., AC/Ni-CF, AC/CC) before and after AC coating process was measured by a digital balance, and the weight difference (triplicate results) was the actual AC loading amount on the electrode material. The experimental design was listed

in Table 2.2. The voltage was continuously recorded every 5 min by digital multimeter (2700, Keithley Instruments Inc., Cleveland, OH, USA). The polarization test (scan rate: 0.5 mV s^{-1}) was performed by using a potentiostat (Reference 600, Gamry Instruments). The concentration of chemical oxygen demand (COD) was measured using a DR/890 colorimeter (HACH Co., Ltd., USA). Total Coulombs (TC), Coulombic efficiency (CE) and COD removal efficiency (COD_R) were calculated according to the previous work [32, 211].

2.3. Results and discussion

2.3.1 Bare cathode electrode without AC catalysts

The cathode electrode materials were firstly examined without AC catalysts to evaluate base material in the three-electrode cell, including CC, CF and Ni-CF. The LSV results show that the onset potentials for these electrode materials were similar, but the peak absolute current density of Ni-CF (3.87 mA cm^{-2}) was much higher than CC (0.39 mA cm^{-2}) and CF (0.63 mA cm^{-2}) (Figure 2.2A), indicating the greater electron conductivity of Ni-CF than other two materials. The Nyquist plots derived by EIS was used to obtain the charge transfer resistance for different cathode materials (Figure 2.2B), based on the equivalent circuit (EC) model shown in Figure B3. The charge transfer resistance of Ni-CF was 2413.5Ω , much lower than that of CC (3954.2Ω) or CF (3214.9Ω) (Figure 2.2C), indicating the stronger ability of Ni-CF to transfer electrons through the electrode material. It was expected that Ni-CF would have a lower ohmic resistance, because of the conductivity enhancement due to the metallic coating, semi-conductive carbon fibers, and the electrical contacts generated from the fiber networks [212]. Therefore, Ni-CF could be an effective base material for MFC cathode electrode to replace CC.

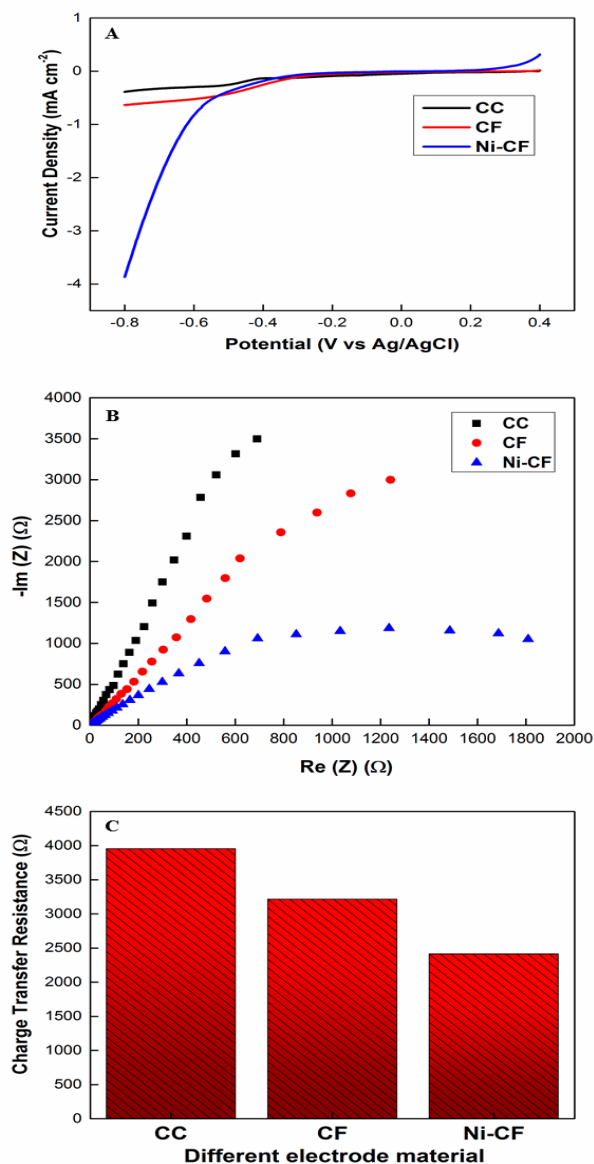


Figure 2.2 Electrochemical analysis of bare electrode materials in the three-electrode cell system, including CC, CF and Ni-CF: A) LSV result; B) EIS Nyquist plot; and C) charge transfer resistance derived from EIS result. Note: CC represents carbon cloth; CF represents carbon fiber; and Ni-CF represents Ni-coated carbon fiber.

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2.3.2 Cathode electrode coated with AC catalysts

Next, the cathode electrodes coated with AC catalysts were examined, including AC/CC, AC/Ni-CF, and pre-treated AC on Ni-CF (W-AC/Ni-CF). The pre-treatment of AC powder by the acidic/basic wash aimed to increase the catalytic performance introduction of oxygen-rich group

by acid washing and activation of AC by basic washing [205]. With the same initial AC coating amount (4 g), the actual loading density of AC on Ni-CF was $8.08 \pm 0.23 \text{ mg cm}^{-2}$ (PTFE excluded out for AC loading density), much greater than that of CC with loading density at $3.61 \pm 0.11 \text{ mg cm}^{-2}$ (Figure 2.3A); this suggests that that Ni-CF could be a better electrode material base with stronger affinity with AC than CC, and such a difference in AC loading would result in different electrochemical performance.

The coated electrodes were examined by using LSV in the three-electrode cell. With potential change until -0.8 V (vs. Ag/AgCl), the current density of AC/Ni-CF was 8.07 mA m^{-2} , much greater than 3.40 mA m^{-2} of AC/CC and 6.00 mA m^{-2} of W-AC/Ni-CF (Figure 2.3B), likely benefiting from its lower charge transfer resistance of 95.1Ω obtained from EIS tests (Figure 2.3C and 3D). For comparison, the charge transfer resistance of AC/CC and W-AC/Ni-CF was 115.3Ω and 187.4Ω , respectively. It was unexpected that W-AC/Ni-CF had the poorer electrochemical performance than AC/Ni-CF. The possible reason for decreased performance of W-AC/Ni-CF was that the acid and alkaline pretreatment on AC under high temperature exposed to the air might have introduced acidic surface oxides on AC itself [205, 213], to make affinity barrier of AC onto Ni-CF, resulting in a much lower AC loading density of $1.89 \pm 0.59 \text{ mg cm}^{-2}$ on the W-AC/Ni-CF (Figure 2.3A). In addition, the increasing oxygen content on carbon surface could increase the electrical resistivity to lower the electrochemical performance [214]. However, the exact reason for lower performance of W-AC/Ni-CF warrants further investigation. The LSV and EIS results have demonstrated that AC/Ni-CF had a stronger ORR catalytic ability than AC/CC.

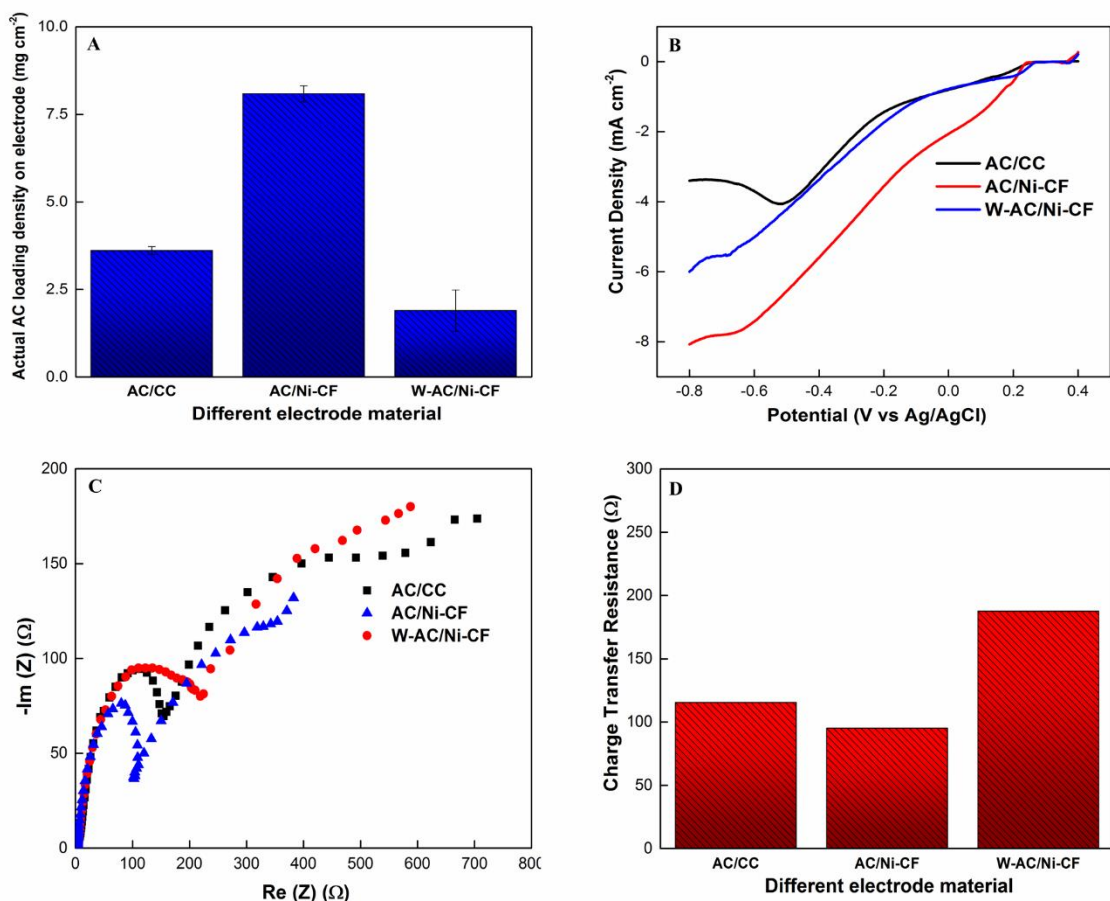


Figure 2.3 Examination of AC coated electrodes in the three-electrode cell system, including AC/CC, AC/Ni-CF and W-AC/Ni-CF: A) actual AC loading density (mg cm^{-2}) on different electrode material applied in the real tubular MFC test (surface area: 170 cm^2); B) LSV result; C) EIS Nyquist plot; D) charge transfer resistance derived from EIS result. Note: AC/CC represents activated carbon coated on carbon cloth; AC/Ni-CF represents activated carbon coated on Ni-coated carbon fiber; W-AC/Ni-CF represents acid/alkaline washed activated carbon coated on Ni-coated carbon fiber. The actual AC loading amount is calculated by excluding PTFE out from the AC/PTFE mixture (AC loading weight (g) = total weight difference (g) before and after coating process $\times (1.92/(1+1.92))$), assuming AC/PTFE mass ratio constant as 1.92).

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To further investigate the electrode performance with bioelectricity generation, the AC coated materials were examined in the tubular MFCs. The batch profiles of current generation with three cathode electrodes are shown in Figure 2.4A. The highest maximum current of 1.7 mA was obtained with AC/Ni-CF, while the other materials generated 1.3 mA (AC/CC) and 1.0 mA (W-AC/Ni-CF), respectively. As a result, the total Coulomb production in a batch was $118.3 \pm 9.8 \text{ C}$

with AC/Ni-CF, much higher than 82.1 ± 11.0 C with AC/CC or 86.8 ± 6.3 C with W-AC/Ni-CF (Figure 2.4B). To ensure that those differences were not due to different reactor preparation, the cathode materials of AC/Ni-CF and AC/CC were exchanged between the MFCs, and the similar current output was obtained, thereby confirming the superior electrochemical performance of AC/Ni-CF (data not shown). COD removal is a key parameter to evaluate MFC performance, especially for its application in wastewater treatment. The cathode electrode materials clearly affected COD removal (Figure 2.4B): the highest COD_R of 74.0 ± 1.6 % was obtained with AC/Ni-CF, followed by 65.6 ± 5.1 % with AC/CC and 61.1 ± 1.0 % with W-AC/Ni-CF. Consequently, Coulombic efficiency (CE) was estimated as 46.6 ± 4.7 % (AC/Ni-CF), 36.4 ± 3.3 % (AC/CC), and 41.4 ± 4.0 % (W-AC/Ni-CF), respectively. Those results suggest that the better electrochemical performance of AC/Ni-CF can also result in better treatment performance. The cost efficiency of the cathode materials was estimated based on the maximum power output. The power density curves show that the maximum power density was 6.50 W m^{-3} with AC/Ni-CF, 4.29 W m^{-3} with AC/CC, and 1.85 W m^{-3} with W-AC/Ni-CF, respectively (Figure 2.5A), confirming the superior electrochemical performance of Ni-CF. The cost efficiency of AC/Ni-CF was $299.0 \text{ mW } \$^{-1}$, much higher than that of AC/CC ($151.7 \text{ mW } \$^{-1}$) or of W-AC/Ni-CF ($15.9 \text{ mW } \$^{-1}$) (Figure 2.5B). Furthermore, the cost efficiency of AC/Ni-CF in this study is also much higher than generally conventional Pt-catalyzed cathode, reported as $5.8 \sim 10.2 \text{ mW } \$^{-1}$ [215-217]. Therefore, Ni-CF was proved to be an effective cathode electrode material alternative to CC with higher electricity generation and cost efficiency.

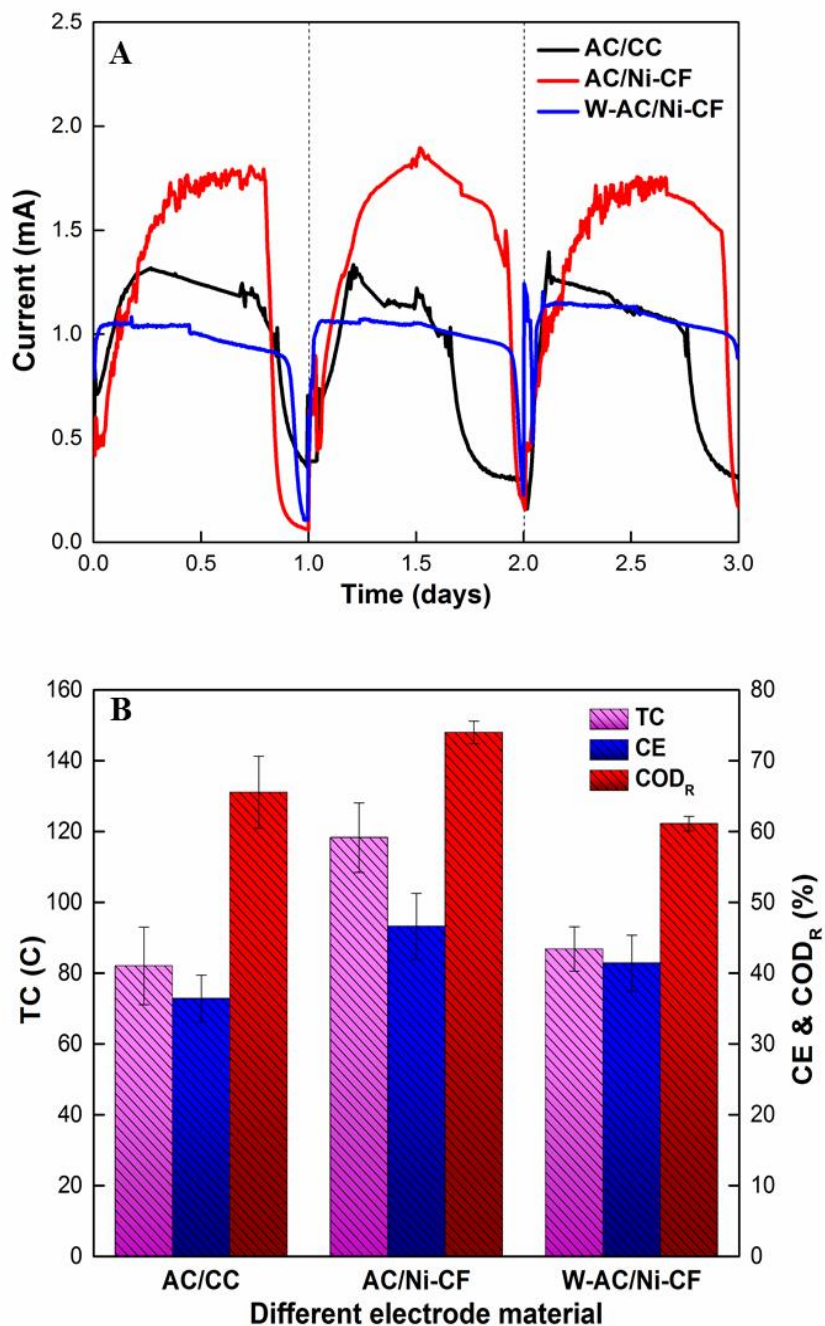


Figure 2.4 The MFC tests of the AC coated cathode electrodes, including AC/CC, AC/Ni-CF and W-AC/Ni-CF: A) batch current profile; and B) average TC, CE and COD_R. AC/CC represents activated carbon coated on carbon cloth; AC/Ni-CF represents activated carbon coated on Ni-coated carbon fiber; W-AC/Ni-CF represents acid/alkaline washed activated carbon coated on Ni-coated carbon fiber.

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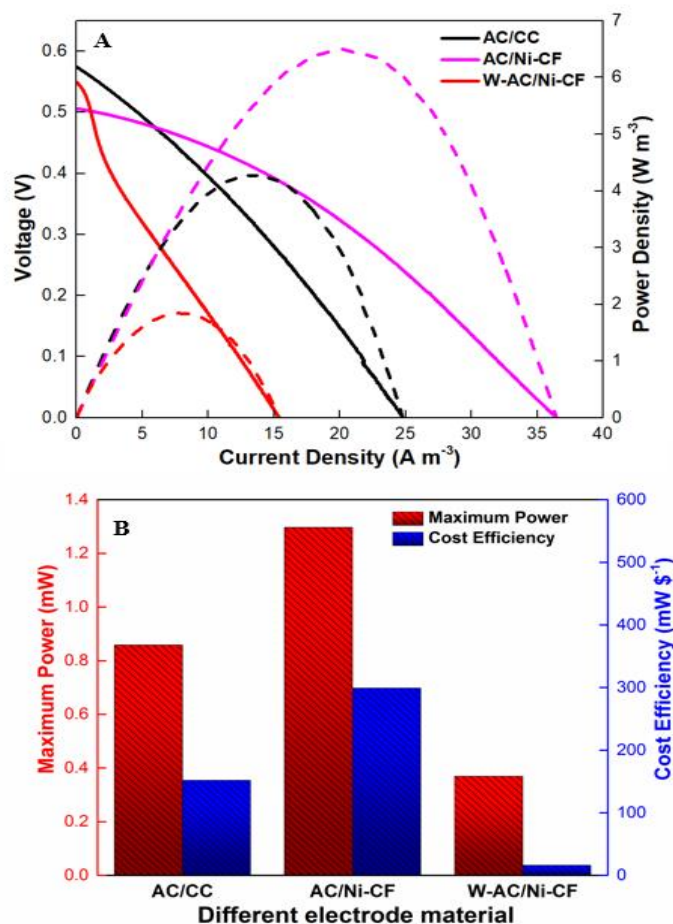


Figure 2.5 Polarization curve of the MFC (A) and cost efficiency analysis (B) of AC coated cathode electrodes, including AC/CC, AC/Ni-CF and W-AC/Ni-CF. AC/CC represents activated carbon coated on carbon cloth; AC/Ni-CF represents activated carbon coated on Ni-coated carbon fiber; W-AC/Ni-CF represents acid/alkaline washed activated carbon coated on Ni-coated carbon fiber.

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2.3.3 Effects of AC catalyst coating amount

The AC/Ni-CF was further investigated with different AC coating amount for electrochemical performance and cost efficiency. The actual AC loading density on the electrode increased with successful loading density from 0 to $21.15 \pm 0.21 \text{ mg cm}^{-2}$ with increasing initial coating amount from 0 g to 10 g (Figure 2.6A). The following presentation of the data will be based on “initial AC coating amount of AC” during the experiment, and the “actual loading amount of AC” indicated

the amount of actual AC loaded on the electrode. LSV tests show that the current density increased with increasing the initial AC coating amount from 0 to 4 g and reached the maximum current density of 9.30 mA cm^{-2} (potential at -0.8 V vs Ag/AgCl) with 4 g. A higher initial AC coating amount than 4 g actually decreased the current density, and the highest initial AC coating amount of 10 g had a very low current density of 2.32 mA cm^{-2} (Figure 2.6B). Such a relationship between current density and initial AC coating amount could be due to insufficient AC catalytic ability or supply of terminal electron acceptors, affected by AC coating. In the first half range of initial AC coating amount within 0-4 g, increasing AC coating amount can actually increase actual loading amount to enhance ORR catalytic ability because of more catalysts. Further increasing AC coating amount might have caused the overload of AC and PTFE mixture, which resulted in excessive filling material inside the interconnecting network and created a barrier for oxygen transfer to the active sites, thereby impeding the electrochemical reduction of oxygen [218]. In addition, a higher amount of PTFE on Ni-CF could also increase the hydrophobicity of the cathode electrode, which would impede the proton supply to the cathode electrode and thus limit current generation [219, 220]. The excessive amounts of PTFE binder will increase the electrode resistance [213], and this was proved by EIS tests. The Nyquist plot for Ni-CF with different amounts of AC coating amount is shown in Figure 2.6C. The charge transfer resistance exhibited an opposite trend to that of current density: the lowest resistance of 181.4Ω was obtained with initial AC coating amount at 4 g, while both lower and higher coating amount gave higher resistance, for example 326.3Ω with initial AC coating amount at 0 g and 294.3Ω with initial AC coating amount at 10 g (Figure 2.6D). The results of both LSV and EIS tests demonstrate that there is an optimal coating amount (e.g., initial coating amount of AC at 4 g in this study) to guarantee optimal actual loading amount on the electrode, and higher or lower AC loading will decrease current generation via decreasing ORR catalytic ability or (possibly) supply of electron acceptors.

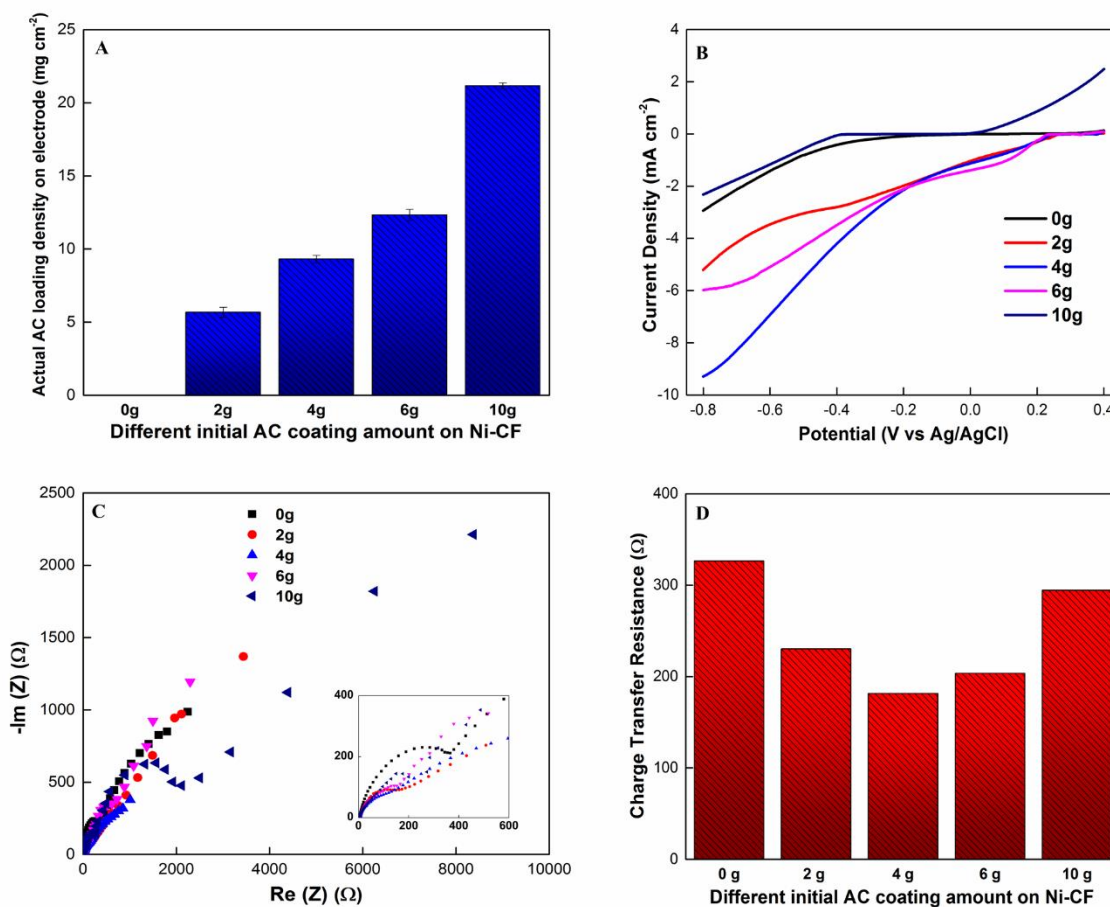


Figure 2.6 Examination of different initial AC coating amount on Ni-CF in the three-electrode cell system: A) actual AC loading density (mg cm⁻²) on Ni-CF applied in the real tubular MFC test (surface area: 170 cm²); B) LSV result; C) EIS Nyquist plot; and D) charge transfer resistance derived from EIS result. Note: “0, 2, 4, 6, 10 g” represent the initial weight of AC added to the solution for coating Ni-CF. The actual AC loading amount is calculated by excluding PTFE out from the AC/PTFE mixture (AC loading weight (g) = total weight difference (g) before and after coating process*(1.92/(1+1.92)), assuming AC/PTFE mass ratio constant as 1.92).

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The MFC test of the coated electrodes confirmed the finding of electrochemical tests that the highest current output was obtained with the initial AC coating amount at 4 g, while the current output decreased to a lower level with higher initial AC coating amount at 10 g (Figure 2.7A). Accordingly, the highest total Coulomb production of 84.8 ± 2.8 C was also obtained with the initial AC coating amount at 4 g, which achieved 74.2 ± 2.6 % of COD removal and 33.7 ± 4.2 % of CE, higher than those with other initial AC coating amount (Figure 2.7B). Based on the maximum power density of 8.70 W m^{-3} (Figure 2.8A), the cost efficiency with the initial AC

coating amount at 4 g was estimated to be $399.4 \text{ mW } \$^{-1}$, significantly higher than $79.4 \text{ mW } \$^{-1}$ with initial 0 g or $95.5 \text{ mW } \$^{-1}$ with initial 10 g (Figure 2.8B). It should be noted that the difference in cost efficiency with initial AC coating amount at 4 g between this section and the section 3.2 was due to different power output affected by MFC operation, and the conclusion of comparison is valid in both cases. Those results have collectively demonstrated that the initial AC coating amount at 4 g appeared to achieve optimal actual loading amount on Ni-CF in this study.

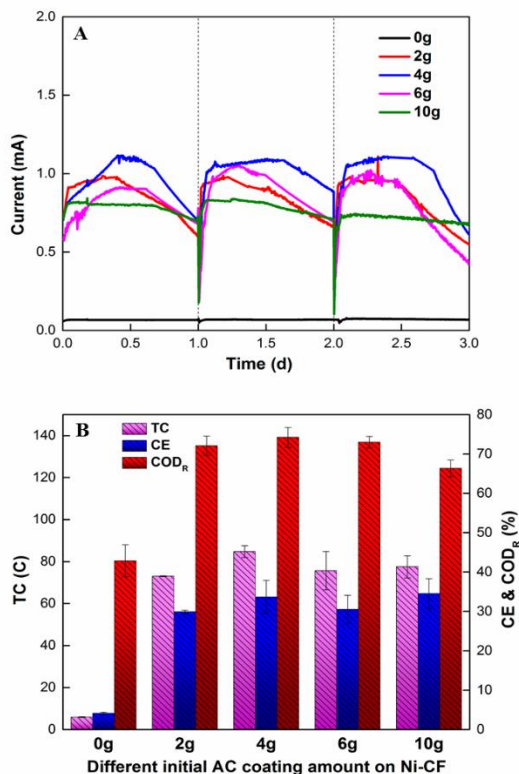


Figure 2.7 The MFC tests of the Ni-CF electrode initially coated with different amounts of AC catalysts: A) batch current profile; B) TC, CE and COD_R. Note: “0, 2, 4, 6, 10 g” represent the initial weight of AC added to the solution for coating Ni-CF.

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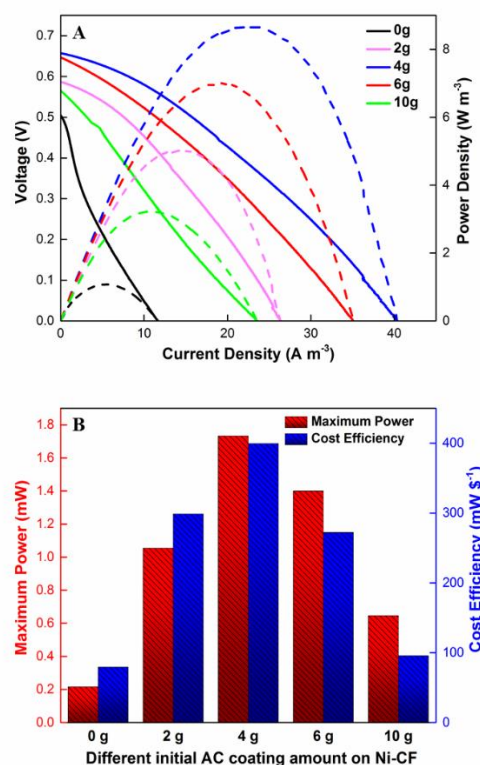


Figure 2.8 Polarization curve of the MFC (A) and cost efficiency analysis (B) of Ni-CF initially coated with different amounts of AC catalysts. Note: “0, 2, 4, 6, 10 g” represent the initial weight of AC added to the solution for coating Ni-CF.

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2.3.4 Perspectives

The outstanding performance of Ni-CF in terms of electricity generation and cost efficiency suggests that it is a feasible approach to explore “new cathode electrode materials” from commercially available products. Many conductive materials have been developed for various purposes, but not specifically for electrode applications, for example Ni-CF is originally designed for electromagnetic shielding and light strike protection for aircraft. Examining those products and selecting optimal ones for MFC applications will avoid the need for scaling up manufacturing processes, which is always a key challenge for newly synthesized materials.

Further applications of Ni-CF for MFC cathodes will need to address several fundamental and practical questions. First, the mechanism of the improved AC loading with Ni-coated carbon fiber surface is not fully understood and the relevant surface chemistry should be investigated further.

This is very important to achieve optimal loading, because our results have demonstrated that AC loading played a key role in electricity generation and thus cost efficiency. Second, because of the importance of AC loading, it will be of strong interest to explore whether the actual AC loading can be further increased without sacrificing MFC performance. A key factor here is the function of binding agent. PTFE, though at a relatively low cost, can cause problems of oxygen transfer when being applied in an excessive amount. Alternative binding agents that can be electrically conductive and create porous conditions to facilitate oxygen transfer will be of great interest. The popular binding agent – Nafion may not be suitable for MFC applications because of its high cost. Third, pretreatment of Ni-CF to enhance AC affinity can be further explored. Understanding of surface chemistry during AC coating process onto Ni-CF will help with formulating an effective pretreatment method. Fourth, the role of Ni in ORR catalysis should be further studied. Our data demonstrate that Ni-CF had a lower charge transfer resistance than CF, indicating potential ORR catalysis with Ni. However, the charge transfer resistance of Ni-CF was an order of magnitude higher than that of AC/Ni-CF, suggesting that the major ORR catalysis was carried out by AC, instead of Ni. Thus, investigation of Ni for its ORR catalysis may further improve the benefits of using Ni-CF as MFC cathode electrode materials. Fifth, other earth abundant metals (e.g., Mn, Fe) are also proved to be prospective alternative cathode catalysts, and addition of these metals on the cathode material using certain techniques (e.g., hot-pressing, electrodeposition) deserves further consideration to improve property of AC/Ni-CF for better MFC performance [200, 221]. Last but not the least, the application of Ni-CF in MFCs must be examined with a long-term operation for its stability in life-cycle analysis, since the AC loading is possible to gradually degrade after long-term operation and the MFC performance may differ under various AC coating amount on Ni-CF.

2.4 Conclusions

In this study, Ni-CF has been demonstrated as an effective cathode electrode material alternative to carbon cloth with superior performance in both electricity generation and cost efficiency. The improved performance benefited from both Ni coated surface and a higher loading of AC catalyst on Ni-CF, though the exact mechanism for enhanced AC affinity on Ni-CF warrants further investigation. The acid/alkaline pretreated AC powder exhibited a lower affinity to Ni-CF, resulting in worse performance than AC/Ni-CF. It was found that the initial AC coating amount at 4 g would be helpful to achieve optimal loading amount on Ni-CF; a lower initial coating amount

could result in insufficiently actual loading amount of AC catalyst and thus a lower ORR catalytic ability, while a higher loading might have blocked the supply of terminal electron acceptors – oxygen. The success of employing Ni-CF as a cathode electrode material in MFCs suggests that exploring suitable electrode materials from commercially available products that are not designed for electrodes could avoid scaling up manufacturing processes, although several fundamental and practical problems need to be further investigated.

Chapter 3 Effective Control of Biohythane Composition through Operational Strategies in an Innovative Microbial Electrolysis Cell (MEC)

(This section has been published as: Shuai Luo, Akshay Jain, Anibal Aguilera, Zhen He. Effective control of biohythane composition through operational strategies in an innovative microbial electrolysis cell. Applied Energy 206 (2017): 879-886.)

3.1 Introduction

Fossil fuels (e.g., gasoline, diesel, and compressed natural gas) are commonly recognized for their potential problems of limited storage, strong greenhouse effect (e.g., emission of carbon dioxide) and environmental pollution [8, 222]. Despite the discovery of new fossil fuel like shale natural gas, rapid growth of worldwide population and improved living standard have created increasing demand for energy, depleting natural resources and deteriorating our environment. Thus, alternative and renewable fuel is of strong interest and importance to address global energy crisis. Among various renewable energy sources, bioenergy from wastes is considered sustainable for simultaneous waste treatment and resource recovery [223]. It has been practiced for a long time to produce methane gas from organic wastes via anaerobic digestion. When adding hydrogen gas into methane gas, a new gas fuel - biohythane is created. Biohythane, which is composed of hydrogen (volume ratio: 10-25%) and methane (volume ratio: 75-90 %), is a promising alternative to the traditional fossil fuel because of its higher fuel and heat efficiency [182, 183]. The addition of hydrogen gas can reduce carbon emission, increase burning speed, extend flammability range, and enhance combustion efficiency to assist methane combustion in the car engines [223-226].

Biohythane is mainly produced from the renewable biomass, and the traditional method is to use anaerobic digestion [227]. Both acidogenesis and acetogenesis are involved in hydrogen production, while methanogenesis produces methane [227, 228]. In this way, biohythane can be produced from agricultural and food wastes [227, 229]. However, in a single-phase digestion reactor, the produced hydrogen gas can be easily consumed by the methanogenic populations, and thus the hydrogen production will be inhibited [227]. To solve this problem, a two-phase digester system was employed to separate acidogenesis/acetogenesis and methanogenesis, leading to a higher energy yield than that of the single-phase process [227]. However, a great challenge for hydrogen generation from a single anaerobic digester is the low conversion efficiency, reported to

be only ~ 17% (2 mol hydrogen/mol glucose) [230]. Also, the complex substrates will cause the unpredictable change in the microbial consortia during the fermentation process, and ultimately lead to the significant variation of the hydrogen proportion in biohythane (from 1 to 75%) [224]. This variation presents a great challenge to achieve stable combustion performance in the automobiles [224, 225], because the stable hydrogen proportion is needed to guarantee high combustion efficiency. For example, 16.5% hydrogen content in the biohythane was reported to achieve the highest combustion efficiency [231]. Therefore, a method for producing the biohythane with effective control of hydrogen proportion will be needed.

Microbial electrolysis cells (MECs) have been studied to produce biohythane from organic wastes [180]. MECs are an emerging technology that can use exoelectrogenic bacteria to degrade organic compounds in wastewater and accomplish hydrogen evolution reaction (HER) for hydrogen production with a small applied voltage (0.4-0.8 V) [232-234]. MECs could achieve a higher hydrogen yield than the traditional fermentation with stronger controllability by electricity generation [235]. It was reported that the fermentation effluent could be further treated by an MEC, to finally improve the hydrogen yield up to 81% (9.6 mol hydrogen/mol glucose) [230]. The maximum hydrogen production rate in an MEC could reach as high as $50 \text{ m}^3 \text{ m}^{-3} \text{ MEC reactor day}^{-1}$ [236]. In the presence of methanogens, either in the anode or the cathode of an MEC, methane can be produced from organics (anode) or hydrogen gas (cathode). A single-chamber MEC with Ti/Ru electrodes was reported to increase the hydrogen and methane production by 1.7-5.2 and 11.4-13.6 folds, respectively, over the traditional anaerobic digester [237]. It has been reported that a single-chamber MEC could successfully produce biohythane, with a higher production rate (0.083 L methane L^{-1} reactor and 0.006 L hydrogen gas L^{-1} reactor) than traditional anaerobic digesters (0.064 L methane L^{-1} reactor and 0.005 L hydrogen gas L^{-1} reactor) [180]. However, the drawbacks of this single-chamber MECs are also obvious. For example, the co-existence of methanogens and hydrogen-producing bacteria in the anode would convert the produced hydrogen gas to methane in a long-term operation to reduce the hydrogen proportion [238]. In addition, the prior study did not adopt any methods to stabilize the composition of the produced biohythane, which is needed to meet the commercial requirement (10-25% hydrogen in the biohythane composition).

In this study, an innovative two-chamber MEC with a total liquid volume of 19 L was developed for biohythane production when treating a synthetic domestic wastewater. Two-chamber configuration allows the separated production of methane and hydrogen gas, and ensures healthy hydrogen production without microbial contamination. The operational parameters such as external resistance, anolyte recirculation rate, and hydraulic retention time, were systematically examined to formulate strategies for controlling biohythane composition. The specific objectives of this study were to: (1) demonstrate the feasibility of biohythane production in the proposed MEC system; (2) develop operational strategies to control the ratio between hydrogen and methane; (3) understand energy efficiency (production and consumption) of the proposed MEC system; and (4) provide implications to further scale up MEC systems for biohythane production.

3.2. Material and methods

3.2.1 MEC setup

An MEC reactor ($53.5 \times 7.6 \times 50.8$ cm) was constructed with an anode working volume for wastewater treatment at 18 L and a head space at 2.6 L. Unlike typical two-chamber MECs that have the anode and the cathode chambers in a side-by-side arrangement, the present MEC had the cathode chamber(s) inside the anode chamber (Figure 3.1A). Its cathode consisted of five tubular cathode segments, which were constructed with anion exchange membrane (AEM tube made by Membranes International, Inc., GlenRock, NJ, USA) and were in sequentially hydraulic connection (the total cathode working volume was 1 L). The AEM tubes were wrapped with carbon brush (Gordon Brush Mfg. Co. Inc., Commerce, CA, USA) that acted as the anode electrodes, and inside the AEM tubes there was one piece of carbon cloth (31 x 11 cm) functioning as the cathode electrodes, supported with stainless mesh and connected via titanium wire (Figure 3.1B). The carbon cloth was coated with activated carbon (5 mg cm^{-2}) and platinum (0.1 mg cm^{-2}) as the catalysts for HER. The hydrogen gas from the cathode was collected by using water replacement method (Figure 3.1C), and the methane gas from the anode was collected by a plastic gas bag. The whole experimental setup is shown in Figure 3.1D.

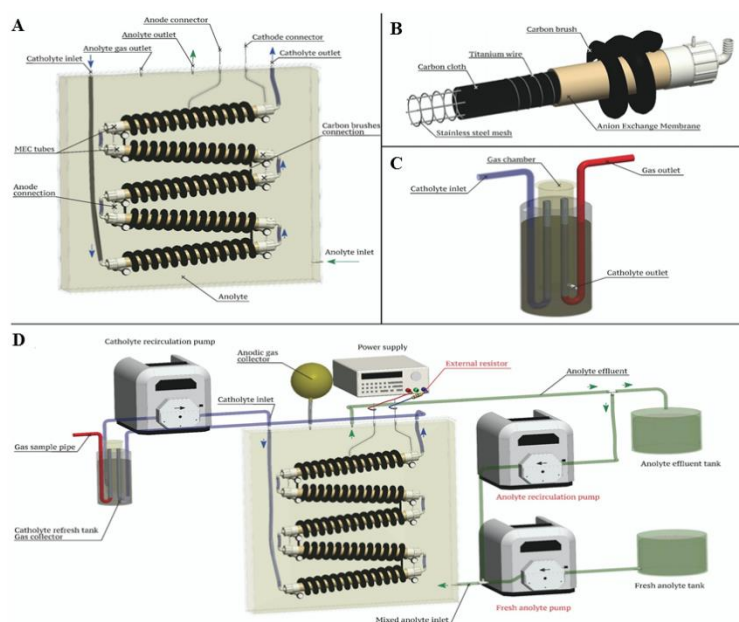


Figure 3.1 Schematic diagram of the scale-up MEC in this study: (A) structure and components of the MEC reactor; (B) internal structure of the cathode and installment of carbon brush (anode material) surrounding cathode; (C) cathode gas collection; and (D) the whole setup of the experiment.

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3.2.2 MEC operation

The MEC anode was inoculated with anaerobic sludge (Peppers Ferry Regional Wastewater Treatment Authority, Radford, VA), and continuously fed with synthetic anolyte to simulate the domestic wastewater (low-strength wastewater [239]) that contained (per L of DI water): 0.17 g sugar (to achieve a COD concentration of $\sim 200 \text{ mg L}^{-1}$, granular sucrose); NH_4Cl , 0.15 g; NaCl , 0.5 g; MgSO_4 , 0.015 g; CaCl_2 , 0.02 g; NaHCO_3 , 0.5 g; and trace element, 1 mL [210]. The anolyte influent had pH of ~ 7.5 and conductivity of $\sim 1.7 \text{ mS cm}^{-1}$. The rates of the anolyte influent and anolyte recirculation were controlled by two peristaltic pumps (MasterFlex L/S, Model No.7524-50, Cole-Parmer Instruments Company, IL, USA). The cathode was operated in a batch mode, in which 2 L catholyte in an external container was refreshed every 12 h (namely one batch cycle). The fresh catholyte contained (per L of DI water): 0.0075 mL concentrated hydrochloric acid to create the pH of 4 for the entire 3 L catholyte after every catholyte refreshment, and 3 g NaCl to guarantee the catholyte conductivity at $\sim 5 \text{ mS cm}^{-1}$ for sufficient ionic conductivity to facilitate production of hydrogen gas. The catholyte was recirculated at 50 mL min^{-1} . An external voltage

of 0.8 V was supplied to the MEC reactor by a power supply (CSI3644A, Circuit Specialists, Inc., Mesa, AZ, USA), and an external resistance (varied) was placed between the negative pole of power supply and cathode electrode to control the current through the MEC system. The MEC reactor was operated at room temperature. The experiment examined the anolyte recirculation rates (0, 200, 500, and 800 mL min⁻¹) at a HRT of 24 h under the external resistance of 1 Ω , external resistance (1, 5, 10, 50, 100 Ω) at a HRT of 24 h and recirculation rate of 800 mL min⁻¹, and HRT (12, 18 and 24 h under the anolyte flow rates at 25.0, 16.7 and 12.5 mL min⁻¹, respectively) at a recirculation rate of 0 and 800 mL min⁻¹ and external resistance of 1 Ω . The summary of experimental design was shown in Table C1.

3.2.3 Measurements

The voltage on the external resistance was recorded every 5 min by a digital multimeter (2700, Keithley Instruments Inc., Cleveland, OH, USA). The pH and conductivity of the anolyte influent and effluent, and the catholyte at the start and end of one batch cycle, were measured by a pH meter (Oakton Instruments, Vernon Hills, IL, USA), and a conductivity meter (Mettler-Toledo, Columbus, OH, USA), respectively. The concentration of chemical oxygen demand (COD) was measured using a DR/890 colorimeter (HACH Co., Ltd., USA) according to the manufacturer's instruction. The produced gas was measured for volume by using a syringe every 12 h. The contents of methane in the anodic produced gas and hydrogen gas in the cathodic produced gas were analyzed by a Shimadzu GC-14A gas chromatograph (GC) equipped with a thermal conductivity meter (TCD), with helium as the carrier gas. To remove carbon dioxide, the produced gases from the anode and the cathode were mixed together and went through an absorption bottle that was filled with 50 mL 0.1 M KOH; the purified biohythane was measured by the GC again to confirm the purity and the gas proportion of methane and hydrogen.

3.2.4 Calculations

Coulombic efficiency (CE, %) was calculated based on actual total Coulombs (TC) accumulation divided by theoretical Coulombs produced from the degraded organics according to a previous study [28]. COD_R represented the removal efficiency of COD (%) derived from the removed COD versus total COD in the MEC influent. The overall cathodic H₂ efficiency (OC, %) was calculated

to reflect the efficiency of HER based on the prior study [240]. The net energy recovery (NER) (kWh day⁻¹) was calculated according to Eq. 3.1:

$$\text{NER} = (\text{Energy gain } (E_{gas}) - \text{Energy consumption } (E_{power} + E_{pump})) \cdot 0.000278 \quad (\text{Eq. 3.1})$$

where the number “0.000278” represents the conversion factor for the unit from kJ day⁻¹ to kWh day⁻¹ for NER.

The energy generation from biogas (E_{gas}) (kJ) was obtained from the sum of methane (E_c) (kJ) and hydrogen gas (E_h) (kJ) produced in a day (two cycles), according to Eq. 3.2 (assuming in 1 atm pressure):

$$E_{gas} = \eta(n_{CH_4} \cdot \Delta H_{CH_4} + n_{H_2} \cdot \Delta H_{H_2}) = \eta \frac{V_{anode} \cdot \varphi_{CH_4} \cdot \Delta H_{CH_4} + V_{cathode} \cdot \varphi_{H_2} \cdot \Delta H_{H_2}}{R_g T} \quad (\text{Eq. 3.2})$$

where n_{CH_4}/n_{H_2} is the number of moles (mol) of ratio of methane/hydrogen; ΔH_{H_2} is the energy content based on the heat of combustion for hydrogen gas (upper heating value) (285.83 kJ mol⁻¹) and ΔH_{CH_4} is the energy content of methane (891 kJ mol⁻¹) [241]; $V_{anode}/V_{cathode}$ represents the total gas volume (L) obtained from anode/cathode in a day (two cycles); $\varphi_{CH_4}/\varphi_{H_2}$ stands for the proportions of methane and hydrogen gas in collected treated gas in anode and cathode respectively; η represents the actual thermal combustion efficiency from the biohythane for gas engine (η assumed as 0.35 in this study based on a previous work [242]); T is the absolute temperature at room (298 K); R_g is the gas constant (0.08206 L atm K⁻¹ mol⁻¹).

The details for the energy consumption of power supply (E_{power}) (kJ) and the pumping (E_{pump}) (kJ) in a day are explained in the following content. Energy consumption derived from power supply and pumping. The calculation equation for energy consumption of power supply (E_{power}) (kJ) in a day (two cycles) was $E_p = 0.0864 \cdot I \cdot U_p$, where I is the current (mA) through the power supply; U_p is the voltage (V) supplied by the power supply for the MEC system; the number “0.0864” represents the conversion factor for the unit from milliwatt (mW) to kJ day⁻¹ for E_{power} . The energy consumption derived from the pumping (E_{pump}) (kJ) in a day (two cycles) calculated based on the Equation 3.3 based on previous works [243, 244]:

$$E_{pump} = 86.4 \cdot \gamma \cdot (2 \cdot Q_1 \cdot H_1 + Q_2 \cdot H_2 + Q_3 \cdot H_3) \quad (\text{Eq. 3.3})$$

where Q_1 , Q_2 and Q_3 are flow rates ($\text{m}^3 \text{s}^{-1}$) of anolyte influent, anolyte recirculation and catholyte recirculation, respectively ($Q_3 = 8.33 \times 10^{-7} \text{ m}^3 \text{s}^{-1}$); H_1 , H_2 and H_3 represent the hydraulic pressure head loss (m) of three pumps (H_1 , H_2 and $H_3 = 0.03$, 0.03 and 0.04 m, respectively); γ is 9800 N m^{-3} ; the number “86.4” represents the conversion factor for the unit from Watt (W) to kJ day^{-1} for E_{pump} ; the reason to add coefficient “2” for Q_1 was that another pump head was applied to take the anolyte effluent with the same flow rate as the anolyte influent, to maintain the same volume and pressure in the head space all the time and guarantee the accuracy for anodic gas measurement.

Efficiency of energy recovery (EER, %) was calculated based on the previous reference [241], dividing NER by the total energy content in the added substrate in a day, with Equation 3.4 shown below:

$$\text{EER} = \frac{\text{NER}}{n_s \cdot \Delta H_s} \quad (\text{Eq. 3.4})$$

where n_s represents the number of moles of substrate added into the MEC system in a day (n_s) (assuming sucrose as substrate and the ratio of COD/sucrose as 1.17 in this study based on preliminary measurement); ΔH_s represents the heat of combustion of the substrate (glucose: 2888 kJ mol^{-1} [241]).

Calculation of carbon balance could reflect the distribution of carbon inside the MEC reactor. The calculation of carbon balance was based on COD equivalent in a 12-hr batch cycle, with details shown in Equation 3.5:

$$\text{COD}_{in} = \text{COD}_{eff} + \text{COD}_{\text{CH}_4} + \text{COD}_e + \text{COD}_{und} \quad (\text{Eq. 3.5})$$

where COD_{in} and COD_{eff} represents the total COD equivalent mass input and output (g) in a 12-hr cycle; COD_{CH_4} represents the COD equivalent mass (g) in the form of anodic CH_4 (assuming 1 mol CH_4 coming from 64 g COD and thus 0.35 L CH_4 produced from one gram COD at standard temperature and pressure [245]); COD_e represents the equivalent COD mass conversion (g) to electricity, which derives from the multiplication of total amount of removed COD from the anolyte influent (g) and CE (%) in a 12-hr cycle; COD_{und} represents the remaining undetected COD (g), including carbon content in dissolved CH_4 in the anolyte, bacterial uptake and produced CO_2 .

Statistical analysis of variance (ANOVA) was carried out using R studio software, to test the impact of the difference of input factors (i.e., HRT, recirculation rate and external resistance) on the system outputs, such as the production rate of methane ($\text{m}^3 \text{ day}^{-1}$) and hydrogen gas ($\text{m}^3 \text{ day}^{-1}$), CE (%), OHR (%), NER (kWh day^{-1}) and EER (%) [246], with significance for $\alpha = 0.05$ (p -value indicates the possibility to make first type statistical error; α is the level of significance).

3.3 Results and discussion

3.3.1 Performance of the scaled MEC

The initial performance of this scaled MEC was investigated under the condition of HRT 24 h, recirculation rate of 0 mL min^{-1} , and external resistance of 1 ohm. Current generation was very stable and the peak current was due to the change of catholyte that introduced more protons (Figure 3.2A). As expected, the catholyte pH increased from 4.0 to 11.1 within a 12-h operation cycle (Figure C1), and this high pH would inhibit the current generation due to the high cathodic overpotential [247]. In the mixed gas produced from the anode and the cathode, carbon dioxide contributed to 82.7% of the total gas volume at a production rate of $1,273 \text{ mL day}^{-1}$ (Figure 3.2B). It is much higher than that of a typical anaerobic digestion, in which carbon dioxide is about 30-40% of biogas. This is because that methanogenesis is not a dominant process in the anode, where electricigens and other oxidation processes can consume organics and produce carbon dioxide. In addition, the bicarbonate content (0.5 g L^{-1}) in the anolyte can also release carbon dioxide. When the KOH treatment was employed to remove carbon dioxide, the treated biohythane contained 34.3 % hydrogen gas ($91.69 \text{ mL day}^{-1}$) and 65.7% methane ($175.25 \text{ mL day}^{-1}$) (Figure 3.2B). The total energy production based on the treated biohythane was $0.73 \pm 0.06 \text{ kWh day}^{-1}$, greater than the energy consumption of $0.34 \pm 0.01 \text{ kWh day}^{-1}$ by the MEC operation, resulting in a NER of $0.39 \pm 0.06 \text{ kWh day}^{-1}$ (Figure 3.2C). This indicates that the present MEC could potentially achieve energy positive with biohythane production. A carbon balance was constructed to better understand the fate of organic carbon in this system (Figure 3.2D). It was found that 14.38% of carbon was converted to methane gas, and only 0.38% was used for electricity generation (which was to drive hydrogen production). The anode effluent still contained 44.98% of carbon, while 40.26% of the input carbon was removed by undefined processes, which may include bacterial uptake, dissolved methane in the MEC effluent, and other microbial oxidation processes.

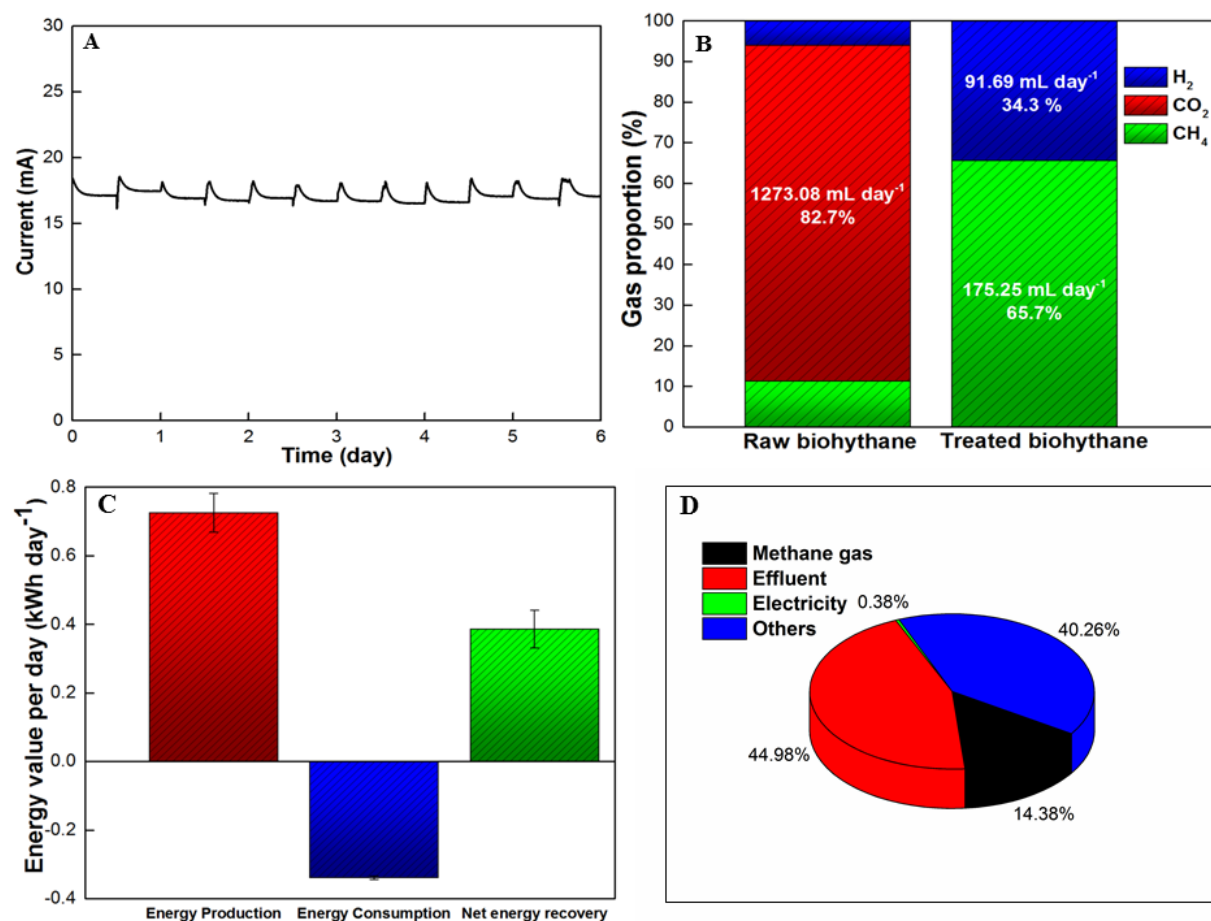


Figure 3.2 Initial performance of the MEC under a condition of HRT 24 h, recirculation rate of 0 mL min⁻¹, and external resistance of 1 ohm: (A) current generation; (B) composition of the collected gas before and after KOH treatment; (C) energy balance analysis; and (D) carbon distribution in the MEC.

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The hydrogen production rate in the present MEC was significantly lower than some reported in literature. However, it should be noted that high production rates are usually obtained from very small scale MECs [236, 248]. Table C2 shows several large-scale MEC systems (> 10 L volume) and one can see that in general large-scale MECs cannot achieve high production rates. This is related to reactor design and operation, and the fact that the optimized features obtained from small reactors cannot be directly applied to large systems. For example, the distance between two electrodes is usually very small in small-scale reactors, resulting in small internal resistance, but this can hardly be maintained in a large-scale system because of the need for volumetric scaling up. In addition, use of actual wastewater or complex substrates will reduce production rates.

Nevertheless, the hydrogen production performance of the present MEC is within the normal range of large-scale system and further improvement through system optimization will be of critical importance to next-stage development.

3.3.2 Effects of anolyte recirculation rate

The anolyte recirculation rate could strongly affect the mixing condition and thus substrate distribution, and contribute mostly to the energy consumption of the MEC system [249, 250]. To better understand its effect on biohythane production, the anolyte recirculation rates of 0, 200, 500, and 800 mL min⁻¹ were investigated. After biohythane purification via removing carbon dioxide, it was found that the biohythane production rate increased from 0.26 ± 0.03 to 0.64 ± 0.06 L day⁻¹ along the increase of the anolyte recirculation rate from 0 to 800 mL min⁻¹, mainly due to the increase of methane production from 0.18 ± 0.02 to 0.53 ± 0.05 L day⁻¹ (methane percentage in the biohythane rising from 65.6% to 83.9%) (Figure 3.3A). Such an increase might have benefited from that: 1) a higher anolyte recirculation rate provided stronger turbulence to facilitate methane release from the dissolved fractions; and 2) efficient dispersion and distribution of substrate and nutrient in the anode accelerated the microbial activity for methane production [251]. A higher anolyte recirculation rate slightly improved the current generation, resulting in an enhanced TC accumulation from 739.1 ± 10.4 C (no recirculation) to 866.5 ± 20.1 C (800 mL min⁻¹). The proportion of hydrogen gas decreased from 34.4% (no recirculation) to 16.1% at 800 mL min⁻¹, falling in the range of the commercial biohythane requirement (10-25%). The proportion of carbon conversion to methane was greatly increased from 14.4 % to 44.2 %, and EER increased from 2.83 ± 0.43 % to 11.23 ± 1.48 % when the anolyte recirculation rate increased from 0 to 800 mL min⁻¹ (Figure 3.3B). The relatively lower EER of this MEC than others using acetate was due to more complex nature of sugar used in this study [241]. In addition, the ratio between the anode electrode size (carbon brush) versus anode liquid volume should be increased to improve the microbial abundance for the substrate conversion to methane and electricity. Furthermore, some generated methane was dissolved in the effluent and lost as other undefined proportion (20-40%) (Figure 3.3B). With a higher recirculation rate, the increase of carbon proportion in methane was strongly related with the decrease of carbon in other undefined proportion, probably from the decrease of the dissolved methane in the effluent due to stronger anolyte turbulence caused by a higher anolyte recirculation rate. Although the increase of the anolyte recirculation had limited improvement on

the CE, TC and COD_R (Figure C2), the energy production significantly increased from 0.72 ± 0.06 kWh day⁻¹ at 0 mL min⁻¹ to 2.01 ± 0.20 kWh day⁻¹ at 800 mL min⁻¹ (Figure 3.3C). In addition, NER increased from 0.39 ± 0.06 kWh day⁻¹ at 0 mL min⁻¹ to 1.52 ± 0.19 kWh day⁻¹ at 800 mL min⁻¹ (Figure 3.3C). The increase benefited from the higher methane production rate. Therefore, a high anode recirculation rate at 800 mL min⁻¹ could increase the biohythane production rate and create more energy benefit despite increased energy input to the recirculation pump.

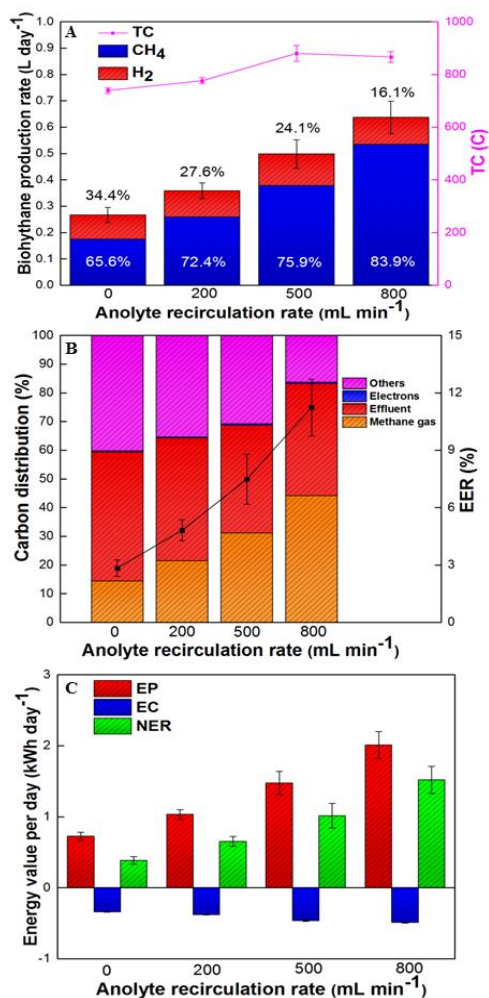


Figure 3.3 Effects of the anolyte recirculation rates on the MEC performance: (A) total coulomb (TC), biohythane production rate and proportions of H₂ and CH₄; (B) carbon distribution conditions and EER; and (C) energy production (EP), energy consumption (EC) and net energy recovery (NER).

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3.3.3 Effects of external resistance

External resistance can strongly affect current generation and thus influence the ratio between hydrogen and methane gas. The external resistance of the MEC was varied at 1, 5, 10, 50 and 100 ohm, while the system was operated with a fixed anolyte recirculation rate of 800 mL min⁻¹ and a HRT of 24 h. As expected, the TC accumulation decreased from 866.5 to 197.5 C with increasing the external resistance from 1 to 100 ohm (Figure 3.4A). The decreased TC resulted in a lower production rate of hydrogen gas, and ultimately reduced the proportion of hydrogen gas in the biohythane composition from 16.1 to 2.9 %. The low proportion of hydrogen gas in biohythane (< 10 %) would make the produced biohythane inapplicable for the commercial application (10-25%). Thus, the external resistance should be below 5 ohm to achieve 10% or greater portion of hydrogen gas in biohythane. Furthermore, the higher external resistance would inhibit the activity of exoelectrogenic bacteria for organic degradation and electricity generation, to finally reduce COD_R, CE and OC and limit the organic removal efficiency and energy recovery (Figure C3A). The carbon distribution analysis further showed a lower wastewater treatment efficiency with the increased external resistance, with a higher percentage of carbon remained in the anolyte effluent (Figure C3B). The only apparent benefit of increasing the external resistance was the reduction of power consumption due to lower current generation ($E_{power} = I \cdot U$, lower I causing lower E_{power}), and thus NER increased from 1.52 ± 0.19 to 1.80 ± 0.09 kWh day⁻¹ (Figure 3.4B). However, the produced biohythane with an excessively low proportion (<10 %) of hydrogen gas under the high resistance would not qualified for commercial applications.

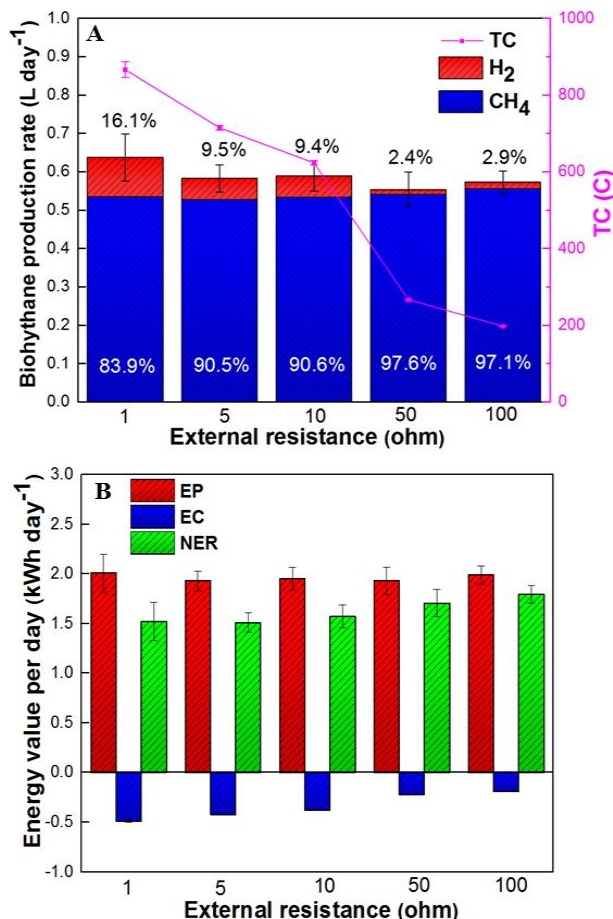


Figure 3.4 Effects of external resistance on the MEC performance (HRT of 24 h and anolyte recirculation rate of 800 mL min⁻¹): (A) biohythane production rate and proportions of H₂ and CH₄; and (B) energy production (EP), energy consumption (EC) and net energy recovery (NER).

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3.3.4 Effects of HRT

HRT is a key operational parameter to biological reactors and can affect organic loading and hydraulic conditions. Three HRTs, 12, 18 and 24 h, were studied under the anolyte recirculation rate of either 0 or 800 mL min⁻¹. The external resistance was kept at 1 ohm. The results showed that the change of the anolyte HRT had a limited impact on the biohythane production (e.g., increased the biohythane production rate only by 0.08 L day⁻¹ with decreasing the HRT from 24 to 12 h, much lower than 0.4 L d⁻¹ by increasing the anolyte recirculation rate from 0 to 800 mL min⁻¹) (Figure 3.5A). At 800 mL min⁻¹, the proportions of methane and hydrogen gas were not

significantly changed, varied at 15-16 % for hydrogen gas, and this was 29-35% at 0 mL min⁻¹ likely because that a shorter HRT might have benefited the anolyte mixing in the absence of the anolyte recirculation. Both COD_R and EER significantly decreased along with the decrease of the HRT. Under the recirculation rate of 800 mL min⁻¹, COD_R and EER decreased from 60.9 and 11.2 % (HRT 24 h) to 42.4 and 6.1 % (HRT 12 h), respectively (Figure 3.5B), likely because of a higher organic loading at a lower HRT. The carbon distribution at the same recirculation (0 and 800 mL min⁻¹) was not significantly varied under three HRTs (Figure C4A). In addition, the decreasing tendency of CE and OC, and stable TC accumulation along the decrease of the HRT further proved that the higher HRT might have inhibited the conversion efficiency from the degraded organic to the electricity generation (Figure C4B). Finally, the increase of NER was limited, only from 1.52 ± 0.19 to 1.75 ± 0.11 kWh day⁻¹ when decreasing the HRT from 24 to 12 h (anolyte recirculation rate of 800 mL min⁻¹) (Figure 3.5C).

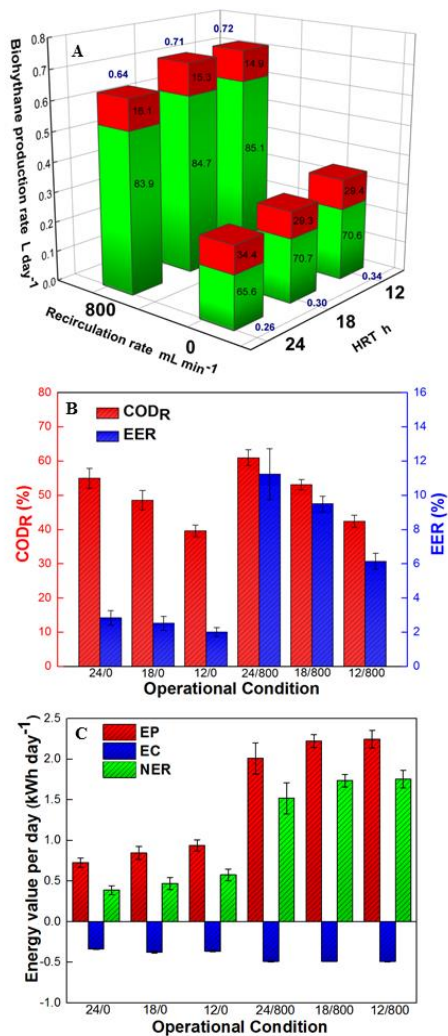


Figure 3.5 Effects of HRT on the MEC performance at two recirculation rates (0 and 800 mL min⁻¹: (A) biohythane production rate and proportions of H₂ (red bar) and CH₄ (green bar) (percentage shown as number, %); (B) energy production (EP), energy consumption (EC) and net energy recovery (NER); and (C) COD_R and EER (%). X-axis of Fig. 5B and C: “A/B” means HRT (A) and anolyte recirculation rate (B).

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3.3.5 Statistical analysis

Understanding the interactions between the input factors and the system outputs (e.g., methane production rate, NER) is important to apply the operational strategies to facilitate MEC development for biohythane production. To reveal the significant difference between different input factors on the outputs, ANOVA was performed and the results are shown in Table 3.1. The data confirmed that the effect of the anolyte recirculation rate on methane production was significant (p -value < 0.05), and thus increasing the recirculation rate was needed to enhance methane production rate. The anolyte recirculation rate also strongly impacted both NER and EER (p -value < 0.05), because the energy content and production rate of methane was much greater than those of H₂ in this MEC. Thus, the recirculation rate deserved sufficient focus as a key component of the control strategy. The various external resistance was the only factor to have significant difference on the production rate of hydrogen gas (p -value < 0.05), and thus the proportion adjustment of hydrogen gas in the biohythane strongly depended on the change of external resistance. HRT would have strong influence on NER and EER (p -value < 0.05), but its impact on hydrogen production was weaker than the anolyte recirculation rate and the external resistance. All three factors also had strong impact for the significant difference of NER and EER (p -value < 0.05), and thus they need to be cooperatively combined together to maximize the energy recovery efficiency.

Table 3.1 The results of ANOVA test to indicate the effect of various inputs factors on the MEC performance during biohythane production.

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Output list	HRT	Recirculation rate	Resistance
CH ₄ production rate (L day ⁻¹)	0.1548	1.0869x10 ⁻⁹	0.3704
H ₂ production rate (L day ⁻¹)	0.0814	0.1989	0.0086
CE (%)	0.2587	0.0098	4.5542x10 ⁻⁵

OHR (%)	0.5105	0.0492	0.0071
NER (kWh day ⁻¹)	0.0205	9.5525x10 ⁻¹⁰	0.0023
EER (%)	3.1542x10 ⁻⁴	1.9730x10 ⁻⁶	0.0242

Note: the numbers in the table boxes are *p*-value; *n* = 12 (number of the experimental groups); α = 0.05.

3.3.6 Perspectives

This study has demonstrated that operating factors could successfully affect biohythane production and composition in an innovative MEC. The results can help us better understand the interactions between operational factors and system performance, towards development of effective control strategies based on either single or multiple factors. This is critically important to a system that is designed to produce multiple products with a desired ratio. To further develop this MEC system, several challenges much be properly addressed. First, continuous elimination of carbon dioxide from the anodic gas will be necessary to refine the biohythane composition, and this separation could consume much energy with a potentially high cost. It was reported that carbon dioxide separation or capture, if scaled-up, could take up 25-40 % of the total energy consumption based on fuel demand in a power plant [252]. The KOH treatment is the temporary treatment strategy in this study, and a method for continuous removal of carbon dioxide will need to be developed with less input of resources. Second, the HER barrier such as cathodic overpotential can limit the production rate of hydrogen gas [253], making it difficult to maintain enough proportion of hydrogen gas in biohythane to meet the commercial requirement. Factors such as external voltage supply, application of various cathodic operational modes (e.g., continuous acid addition into catholyte), catholyte recirculation rate, highly-efficient cathodic catalysts, and ratio of cathode/anode volume warrant further investigation. Third, the COD removal efficiency was not high in the present MEC system, and thus enhancing COD removal will be critical to achieve a functional system for wastewater treatment. This may be accomplished by increasing the biomass quantity and activities. Fourth, actual wastewaters must be studied to examine the influence in actual applications of the MEC system. The potential influence includes wastewater composition, temperature and others such as the presence of toxic compounds [239]. Particularly, the composition and concentration of wastewater will largely affect gas production and thus the economic feasibility of the system. The present study used a synthetic wastewater that mimicked

domestic wastewater, and the use of actual domestic wastewater could reduce the gas yield and productivity. Other types of wastewaters containing a high concentration of organic compounds such as brewery wastewater, waste biomass, and agricultural wastes may be considered as a substrate in this system. In addition, some specific wastewaters could contain much ammonia, which can be possibly recovered in the cathode [70]. Thus, there is a potential to accomplish multiple tasks (recovery of methane gas, hydrogen gas, and ammonia gas) in one MEC system. Last but not least, the unique design of the present MEC system was expected to facilitate system scaling up. To achieve it, the membrane module (containing both anode and cathode electrodes) may be scaled up and installed in a tank that acts as an anode. In this way, the efforts of scaling up will be focused on manufacturing membrane modules and we may take advantage of the existing treatment reactors for modification to MECs. In addition, techno-economic assessment should be performed to validate the system practicability and commercial applications.

3.4 Conclusions

This study has demonstrated that the composition of biohythane could be affected by operational factors, and thus control strategies may be developed based on one or more operational factors. It was showed that the anolyte recirculation rate was a key factor to affect methane production and thus its proportion in biohythane. Hydrogen production was controlled by electricity generation, which was affected mostly by external resistance. To maintain the ratio of hydrogen gas >10% in biohythane, the external resistance should not be larger than 5 ohm. HRT had a minor impact on the biohythane composition, but could significantly affect the organic removal rate. The proposed design could be used to scale up the MEC system for biohythane production. The results of this study will help to develop an efficient MEC system to enhance the sustainable energy recovery from wastewater.

Chapter 4 ^{13}C pathway analysis of biofilm metabolism of *Shewanella oneidensis* MR-1

(This section has been published as: Weihua Guo, Shuai Luo (co-first author), Zhen He and Xueyang Feng. ^{13}C pathway analysis of biofilm metabolism of *Shewanella oneidensis* MR-1. *RSC Advances* 5. 50 (2015): 39840-39843.)

4.1 Introduction

Shewanella oneidensis MR-1 has been extensively studied [254-258], especially for its biofilms, which have extraordinary capability in bioremediation of heavy metals [259-263] and electric power generation [264, 265]. Despite recent discoveries of *Shewanella* metabolism under aerobic and anaerobic conditions using various analytical approaches [266-269], few studies have been accomplished to investigate the metabolic pathway usage in *Shewanella* biofilms. In this study, we applied the ^{13}C pathway analysis, a reliable and well-developed technology [270], to analyze the carbon metabolism of *S. oneidensis* cells derived from biofilm and planktonic growth, respectively. Through the comparison of isotopomer labeling patterns of key proteinogenic amino acids, we found that the C1 metabolism was much more active when growing *S. oneidensis* in biofilms compared to planktonic cells, which could be related to the utilization of C1 metabolites as electron donor by *S. oneidensis* when growing in biofilms. To our best knowledge, this is the first time that the biofilm metabolism of *S. oneidensis* was rigorously determined by isotopomer analysis.

4.2 Methods, results and discussion

S. oneidensis MR-1 was initially grown in shake flasks with minimal medium containing 3.7 mM $[3-^{13}\text{C}]$ sodium L-lactate (Sigma-Aldrich) for two days at 100 rpm, 30°C. It was then transferred to completely fill up a 140 mL sealed bottle reactor and cultivated under oxygen-limited condition (stirrer at the bottom) at 30°C. To grow *S. oneidensis* MR-1 in a biofilm, a carbon cloth (2.5 cm×4.5 cm, Zoltek, Panex[®] 30 Fabric, PW06) was submerged in the medium of the sealed bottle reactor, with titanium mesh (McMaster) to support and titanium wire (Sigma-Aldrich) to bind the cloth tightly on mesh. Duplicate reactors were processed (n=2). When lactate was depleted (<0.01 mM), the minimal medium was refreshed by removing 10 mL medium from the sealed bottle reactor and injecting 10 mL fresh filter (0.22µm pore size) sterilized medium. The final concentration of $[3-$

^{13}C] sodium L-lactate was maintained at $\sim 0.8\text{ mM}$. The biomass of planktonic cells was monitored by OD_{600} using a plate reader (BioTek). The concentrations of lactate and acetate in the sealed bottle reactor were measured by high-performance liquid chromatography (HPLC, Shimadzu), following the method that has been previously developed [271] (Figure 4.1). It was found that lactate was consumed to produce acetate as the sole fermentation byproduct when growing *Shewanella* in the sealed bottle bioreactor. The declining OD_{600} of the planktonic cells in the reactor indicated that more cells would be grown in the biofilms with the replenishment of the medium.

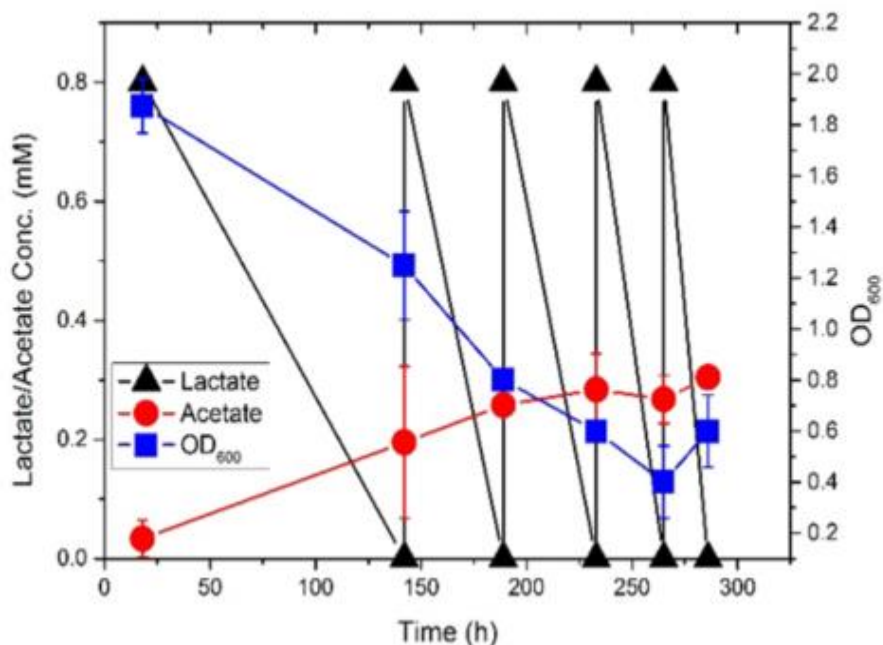


Figure 4.1 Fermentation profile of *S. oneidensis* MR-1 in the sealed bottle reactor.

Reproduced with permission from reference [272].

After five refreshment of the medium, both the planktonic cells and the biofilm cells were collected from the liquid culture and the carbon cloth, respectively, followed by isotopomer analysis of proteiogenic amino acids using a previously developed protocol [273-275]. In general, the biomass was hydrolysed using 6 M HCl (24 h at 100 °C). The amino acids were derivatized in 20 μL of tetrahydrofuran and 20 μL of N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide (Sigma-Aldrich). A gas chromatograph (GC2010, Shimadzu) equipped with a SH-Rxi-5Sil column (Shimadzu) and a mass spectrometer (QP2010, Shimadzu) was used for analyzing the labeling profiles of metabolites. Three types of charged fragments were detected by GC-MS for

Ala, Gly, Ser, Asp and Glu: the $[M-57]^+$ group (containing unfragmented amino acids); and the $[M-159]^+$ or $[M-85]^+$ group (containing amino acids that had lost an α -carboxyl group). For each type of fragments, the labeling patterns were represented by M0, M1, M2, etc., which were fractions of non-labeled, singly labeled, and doubly labeled amino acids. The effects of natural isotopes on isotopomer labeling patterns were corrected by previously reported algorithms[276].

By feeding $[3-^{13}\text{C}]$ lactate as the sole carbon source to *S. oneidensis* under an oxygen-limited culture condition, most alanine molecules were expected to be singly labeled because pyruvate, the direct precursor of alanine, was believed to be mainly synthesized from $[3-^{13}\text{C}]$ lactate (Figure 4.2 and Table 4.1). To our surprise, 34% alanine molecules were found to be non-labeled in the biofilm cells. As a result of the non-labeled pyruvate produced, we also found a significant amount of non-labeled aspartate and glutamate in the biofilm cells, which were synthesized from the futile cycle and the TCA cycle, respectively, by using the non-labeled pyruvate as the precursor. However, it remains unknown that how the non-labeled pyruvate was synthesized during *Shewanella* growth in biofilms.

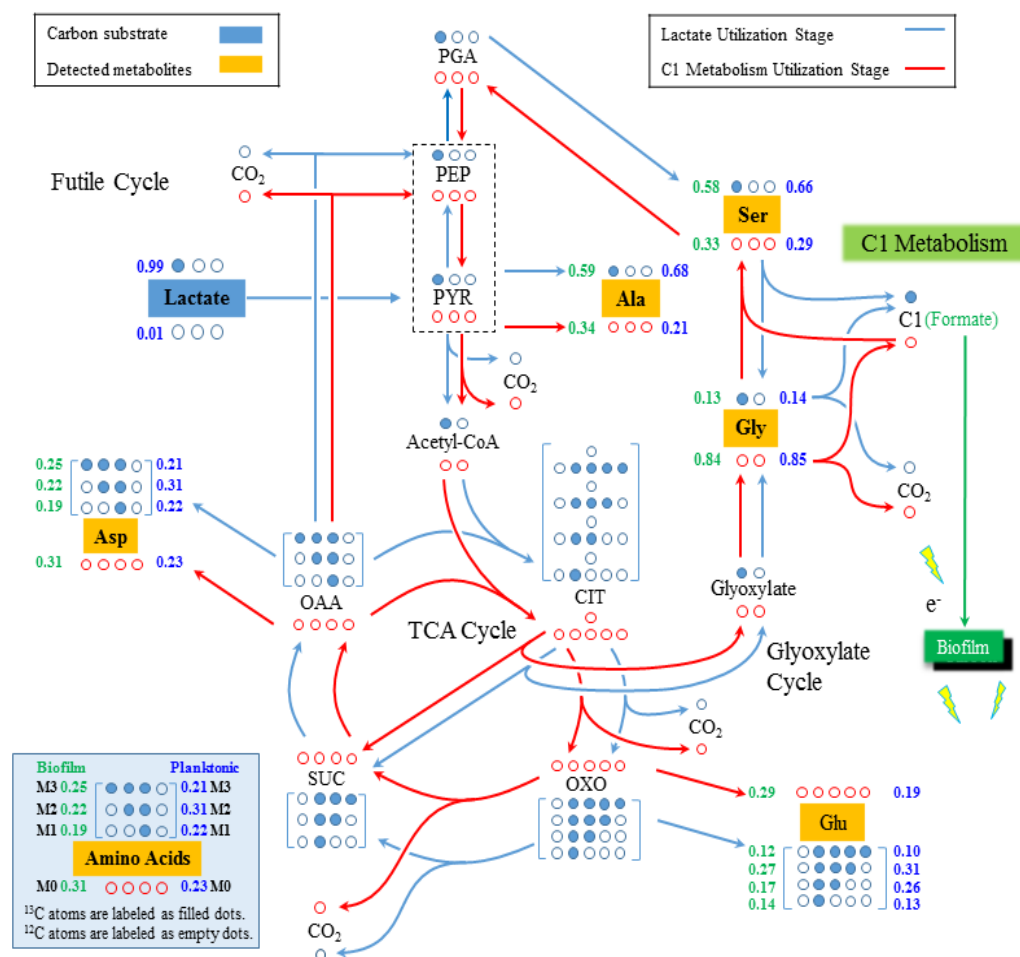


Figure 4.2 Central carbon metabolic pathways of *S. oneidensis* MR-1 from biofilm and planktonic growth. The ¹³C and ¹²C atoms were labeled as filled and empty dots, respectively. The lactate utilization pathways were colored as blue while the C1 metabolic pathways were colored as red. The number on the left side of each isotopomer (green) was the M-57 values of proteinogenic amino acids from the biofilm cells while the number on the right side of each isotopomer (blue) was the M-57 value of proteinogenic amino acids from the planktonic cells. Abbreviations: Ala, alanine; Asp, aspartate; C1, C1 molecule; CIT, citrate; Glu, glutamate; Gly, glycine; OAA, oxaloacetate; OXO, 2-oxoglutarate; PEP, phosphoenolpyruvate; PGA, 3-phosphoglycerate; Ser, serine; TCA Cycle, tricarboxylic acid cycle.

Reproduced with permission from reference [272].

Table 4.1 Mass distribution of detected metabolites for the ¹³C tracing experiments (n = 2)
a.

Reproduced with permission from reference [272].

	Biofilm samples		Planktonic samples	
	M-57	M-159/85	M-57	M-159/85
Ala				
M0	0.34	0.35	0.21	0.22
M1	0.59	0.58	0.68	0.70
M2	0.05	0.07	0.09	0.08
M3	0.01		0.02	
Gly				
M0	0.84	0.87	0.85	0.88
M1	0.13	0.13	0.13	0.12
M2	0.03		0.02	
Ser				
M0	0.33	0.39	0.28	0.29
M1	0.58	0.56	0.63	0.66
M2	0.08	0.06	0.08	0.05
M3	0.01		0.01	
Asp				
M0	0.31	0.33	0.23	0.23
M1	0.19	0.25	0.22	0.31
M2	0.22	0.28	0.31	0.31
M3	0.25	0.14	0.21	0.15
M4	0.02		0.02	
Glu				
M0	0.28	0.29	0.19	0.20
M1	0.14	0.16	0.13	0.16
M2	0.19	0.26	0.26	0.33
M3	0.26	0.27	0.31	0.30
M4	0.13	0.03	0.10	0.02
M5	0.01		0.00	

^a The error in mass distribution from two duplicates was <5%.

To our best knowledge, there is only one pathway, C1 metabolism, which could lead to the synthesis of non-labeled pyruvate when growing with [3-¹³C] lactate [256, 277-279]. Generally, pyruvate could be converted to 3-phosphoglycerate (PGA) through the gluconeogenesis, which is used to produce serine, and further converted into glycine and a C1 molecule (e.g., formate) via C1 metabolism. During the glycine synthesis from serine, the labeled carbon originated from the

[3-¹³C] lactate would get lost as C1 metabolites while glycine became mostly non-labeled, as found in this study (Figure 4.2). Glycine could be continuously divided into CO₂ and C1 molecule, both of which were non-labeled in this study. Considering the high reversibility of the C1 metabolism, the non-labeled C1 metabolites could be incorporated with unlabeled glycine to synthesize non-labeled serine (i.e., reversed C1 metabolism), which would then be used to synthesize the non-labeled pyruvate via serine dehydratase (SDH, EC 4.3.1.17) [280, 281]. This is possible in the present study because we grew *Shewanella* in biofilms with very low concentration of [3-¹³C] lactate (~0.8 mM), which was rapidly consumed for cell growth within 5~10 hours but also produced non-labeled C1 metabolites via the C1 metabolic pathway. Once lactate utilization stage was finished, *Shewanella* growing in biofilms would use the generated C1 metabolites as the secondary carbon source to continue the production of building blocks for cell growth. As a result of the combined effects of lactate utilization and C1 metabolites utilization, the proteinogenic amino acids, such as alanine, aspartate and glutamate, demonstrated a high percentage of non-labeled molecules (34%, 31%, and 28%, respectively). It is also worth noting that although most of detected glycine (84%) is non-labeled, there was 13% single-labeled glycine detected, which could be synthesized from glyoxylate shunt of TCA cycle as that has been discovered in previous studies.[256, 277, 279].

Compared to the isotopomer labeling patterns of the biofilm cells, the percentage of non-labeled proteinogenic amino acids (e.g., Ala, Glu, and Asp) in the planktonic cells of *S. oneidensis* MR-1 was much smaller. For example, only 23% of alanine detected in the planktonic cells was non-labeled while 34% alanine was found to be non-labeled in biofilm cells. Considering the cohesiveness of the biofilm, some cells could be washed out from the biofilm and become the planktonic cells because of the detachment of the biofilm [282, 283]. Indeed, at the end of the fifth medium replenishment, we did observe an increased OD₆₀₀ of the planktonic cells (Figure 4.1), which could be attributed to the washout of the biofilm cells. As a result, the non-labeled amino acids in the biofilm cells were also detected in the planktonic cells. It is also possible that since the substrate (i.e., [3-¹³C] lactate in this study) was more difficult to diffuse into the biofilms, more lactate would be used by the planktonic cells, which makes the lactate utilization metabolism more dominant in the

planktonic cells and leads to smaller percentage of non-labeled pyruvate synthesized from C1 metabolism of planktonic cells.

4.3 Conclusions

The results of this study have important implications to use *S. oneidensis* MR-1 for energy production. For example, it has been well known that by forming the biofilms, *S. oneidensis* MR-1 could generate electricity from organic carbon substrates[265, 284]. The discovery from this study indicated that the C1 metabolite, especially formate, could serve as the electron donor and generate electricity during the C1 metabolism[278]. Considering the direct connection of biofilm cells and carbon cloth, the electron transfer of biofilm cells should be more active compared to planktonic cells, which could lead to active the C1 metabolism in the biofilm cells. The exact pathway of carbon flux during bioelectricity generation will be investigated in the following study. In summary, by applying ^{13}C pathway analysis to investigate microbial metabolism of *S. oneidensis* MR-1 growing in biofilms, we discovered that the activity of C1 metabolism was interestingly higher than that in planktonic cells, which could be related to the utilization of C1 metabolites as electron donor when growing *Shewanella* in biofilms.

Chapter 5 ¹³C pathway analysis for the role of formate in electricity generation by *Shewanella Oneidensis* MR-1 using lactate in microbial fuel cells

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5.1 Introduction

Microbial fuel cells (MFCs) are an emerging technology to convert organics in the wastewater to electrical energy [24, 28]. Electrochemically active bacteria (EAB) serve as effective microbial catalysts to transfer electrons from substrates to solid electron acceptors (e.g., anode electrode) under anaerobic conditions of MFCs [26, 68, 285, 286]. Electron transferring mechanism between EAB and the electrode is one of the most important steps in electricity generation by MFCs, and can be critically influenced by the type of the electron donors that will result in difference in the composition, structure, and metabolism of microorganisms on the anode electrode [287-289]. Therefore, understanding of the impact from electron donors on electricity generation in MFCs is important to improve the efficiency of energy recovery [290-292].

Shewanellaceae is a typical family including several model bacterial species that can generate electricity in MFCs such as *Shewanella oneidensis* MR-1 [289, 293, 294]. *S. oneidensis* MR-1 can transport electrons via intracellular metabolic reactions to complete extracellular electron transfer (EET) pathways [295], via multiple electron-transferring mechanisms including direct contact on the electron acceptor [296], redox reactions of mediators (e.g., flavin) to transfer electrons [297-299], and/or conductive nanowire extension to contact the electron acceptor [300]. Versatile electron transferring abilities allow *S. oneidensis* MR-1 to use multiple organics as electron donors (e.g., lactate, pyruvate, acetate, formate) to process dissimilatory reactions to reduce insoluble metals (e.g. Fe(III), Mn(IV)) and some inorganic ions (e.g., nitrate, nitrite) as electron acceptors [261, 293, 301-303]. Among those electron donors, lactate has been extensively studied. It was found that *S. oneidensis* MR-1 prefers to catabolize lactate because of a series of internally competent enzymatic reactions that utilize the electron donors to obtain energy for growth and

electricity generation [295, 304, 305]. Under anaerobic conditions, including the anaerobic anode of MFCs, lactate can be utilized to release electrons with the involvement of NADH, specific cytochromes, as well as various complexes, and the lactate is further oxidized to acetyl-CoA, and CO₂ or formate [294, 295, 306]. To assess electricity generation efficiency, Coulombic efficiency (CE) and Coulombic recovery (CR) are quantitative indicators to show the fraction of collected electrons through EET versus total ideal electron production [307-309]. It was reported that the lactate-fed MFC inoculated with *S. oneidensis* MR-1 achieved CE at the range about 15-20% [296, 310], where there is still a lot of room to improve electricity generation efficiency.

In addition to lactate, formate is another potentially important electron donor to serve carbon and energy source for *S. oneidensis* MR-1 [293, 295, 311]. *S. oneidensis* MR-1 has NAD⁺-dependent formate dehydrogenase (FDH) to catalyze the formate oxidation to CO₂, and release electrons to complete EET pathway [294]. On the basis of EET started from lactate and pyruvate utilization, the catabolic metabolism of formate with the help of FDH can release and transfer more electrons to specific electron acceptors, which is possibly helpful to improve CE/CR of *S. oneidensis* MR-1 in MFCs [294, 306]. Thus, it is hypothesized that extra formate addition on lactate utilization can boost the electrical output and improve CE/CR. However, formate is seldom studied for its effect on electricity generation of *S. oneidensis* MR-1 in MFCs.

This study aims to investigate the role of formate for the catabolic metabolism and electricity generation of *S. oneidensis* MR-1 in MFC. Particularly, we applied ¹³C tracer experiment, which is a method widely used to facilitate the elucidation of metabolic rewiring in various non-model environmental microorganisms [312-314]. Normally, by feeding the ¹³C labeled substrate into culture system, the carbon flow in the intracellular metabolism is traced to produce labeled metabolites (e.g., proteinogenesis amino acids). By analyzing these metabolites using gas chromatography-mass spectrometry (GC-MS) and the following natural isotopic correction of the raw mass spectrums, the isotopic labeling patterns (e.g., mass distribution vector) would be obtained and could be directly analyzed to reveal the metabolic rewiring of the target strains. Our previous study has, for the first time, applied the ¹³C tracer experiments to a well-designed anaerobic system for revealing the distinct intracellular metabolic behaviors between the biofilm and planktonic *S. oneidensis* MR-1 cells [272]. The ¹³C tracer experiment successfully revealed

the cell metabolisms of *S. oneidensis* MR-1, and a conclusion was made that C1 metabolism such as formate oxidation is strongly related to the electron transferring to the extracellular environment. Therefore, in this study, ^{13}C tracer experiment was applied to help us rigorously analyze the effects of formate on microbial metabolism of *S. oneidensis* MR-1 using lactate for electricity generation in an MFC. As the novel contribution presented in this work, we discovered, for the first time, that the addition of formate in MFCs could indeed synergize with the lactate utilization to improve the electricity generation efficiency, which was achieved by a unique metabolism in *S. oneidensis* MR-1 to decouple cell growth and electricity generation during co-utilization of lactate and formate.

5.2 Methods

5.2.1 Bacterial strains and growing conditions in the MFC

S. oneidensis MR-1 (kindly provided by Dr. Y.J. Tang's lab, Washington University, St. Louis, MO, USA) was initially grown in the shaking flasks with minimal medium containing 5mM sodium L-lactate (Sigma-Aldrich) for two days at 100 rpm and 30 °C [303, 315]. The culture medium was then transferred to completely fill up the autoclaved anode chamber and the control chamber of the MFC (Figure D1), where both chambers were operated under oxygen-limited condition at 30 °C.

5.2.2 MFC setup and operation

A triple-chamber MFC was constructed by connecting three glass bottles together with two CEMs as separators (UltexCMI7000, Membranes International, Inc., GlenRock, NJ, USA) between each set of two bottles (Figure D1). The liquid volume of three chambers was 140 mL/each. The middle chamber served as a cathode chamber, while one bottle containing an anode electrode connected to the cathode electrode was the functioning anode and the other bottle (also containing an anode electrode but under the open circuit condition) was the control chamber. The anode and cathode chambers were sealed with creamy epoxy to create oxygen-limited environment. The anode electrode was a piece of rectangular carbon cloth (2.5 cm $\times$ 4.5 cm PANEX® 30PW06, Zoltek Corporation, St. Louis, MO, USA), and the cathode electrode using the same carbon cloth coated with platinum/carbon as the catalyst for oxygen reducing reaction (0.1 mg Pt cm⁻²). The cathode chamber was continually aerated to provide sufficient oxygen for cathodic reaction. The catholyte

was 50 mM PBS (2.65 g L⁻¹ KH₂PO₄ and 5.35 g L⁻¹ K₂HPO₄), to buffer the catholyte pH to eliminate the limiting effect of cathode.

The MFC was operated under a batch mode with the external resistance of 8.2 Ω. Non-labelled electron donors were first applied, and three conditions were tested: 1) 0.8 mM lactate in 1st - 3rd cycles; 2) 0.8 mM lactate + 0.8 mM formate in 4th - 6th and 14th - 15th cycles; 3) 0.8 mM formate in 7th - 13th cycles. Once the current dropped to the baseline (~ 0.04 mA) in each cycle, the medium in the anode chamber and the control chamber was refreshed by removing 10 mL medium with sterile syringe and then injecting 10 mL fresh filtered (0.22 μm pore size) medium into the chambers. Depending on the substrate, each cycle time period was 16 - 60 hours. To accelerate electron transfer, 0.2 μM riboflavin was added as an electron mediator to the anode and the control chambers at the beginning of the entire experiment (there was no more addition until the end of the experiment).

5.2.3 ¹³C Tracer Experiment

Isotopic-labelled formate as an electron donor was supplied after finishing the cycles with non-isotopic labeled electron donors in the MFC. The combination of 0.8 mM [¹³C] sodium formate (99% purity, Cambridge Isotope Laboratory, USA) and 0.8 mM non-labeled sodium L-lactate was supplied to the anode and the control chambers at the beginning of a new cycle. After nine-cycle tests, both the liquid culture and carbon cloth in the anode and the cathode chambers were extracted to collect the planktonic and the biofilm cells, respectively, followed by the protocol developed previously for the isotopic analysis of proteogenic amino acids[272, 316, 317]. In general, the biomass was hydrolyzed using 6 M HCl (20 h at 100 °C). The amino acids were derivatized in 50 μL of tetrahydrofuran and 50 μL of N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide (Sigma-Aldrich). A gas chromatograph (GC2010, Shimadzu) equipped with a SH-Rxi-5Sil column (Shimadzu) and a mass spectrometer (QP2010, Shimadzu) was used for analyzing the labeling profiles of metabolites. Three types of charged fragments were detected by GC-MS for Ala, Gly, Ser, Asp and Glu: the [M-57]⁺ group (containing unfragmented amino acids); and the [M-159]⁺ or [M-85]⁺ group (containing amino acids that had lost an α-carboxyl group). For each type of fragments, the labeling patterns were represented by M0, M1, M2, etc., which were

fractions of non-labeled, singly labeled, and doubly labeled amino acids. The effects of natural isotopes on isotopic labeling patterns were corrected by previously reported algorithms[318].

5.2.4 Data Measurement

The voltage of the MFC was recorded by a digital multimeter (2700, Keithley Instruments, Inc., Cleveland, OH, USA) with measurement interval of 2 min. The concentrations of formate and lactate at the beginning and the end of each cycle were measured by high-performance liquid chromatography (HPLC) (Shimadzu, Columbia, MD) equipped with an Aminex HPX-87H column (Bio-Rad, Hercules, CA) and refractive index detector (RID, 10A, Shimadzu), with the following program: column temperature, 65°C; mobile phase, 0.5 mM sulfuric acid solution; flow rate, 0.6mL/min. The electrolyte pH was measured by using a pH meter (Oakton Instruments, Vernon Hills, IL, USA).

5.3 Results & Discussion

5.3.1 Combined supply of formate and lactate enhanced current generation

The MFC was operated for three conditions, each of which was operated for at least three repeated cycles and the current profile was recorded (Figure 5.1). The added formate and lactate in the anode chamber was completely consumed in each cycle based on the HPLC results, indicating that the current drop at the end of each cycle was caused by the complete consumption of electron donors (Figure 5.1). In the control reactor that did not have electricity generation (electrodes were under an open circuit condition), the electron donors were also consumed, possibly because of oxygen intrusion from the cathode (with continuous aeration) into the control chamber acting as the electron acceptor[133]. Though the oxygen diffusion through cation exchange membrane (CEM) was very limited[216, 319], the electron donor for each cycle could still be consumed up because of very small amount (~ 0.8 mM) of carbon source added into the control and anode chambers. Electricity generation in the MFC clearly competed for electron donors, and the comparable CR (Coulombic recovery) with other MFC studies of *S. oneidensis* MR-1 indicates that the anode electrode has strong ability to compete with oxygen for the electron donors to collect electrons (data of CR were shown in the following section)[296, 310].

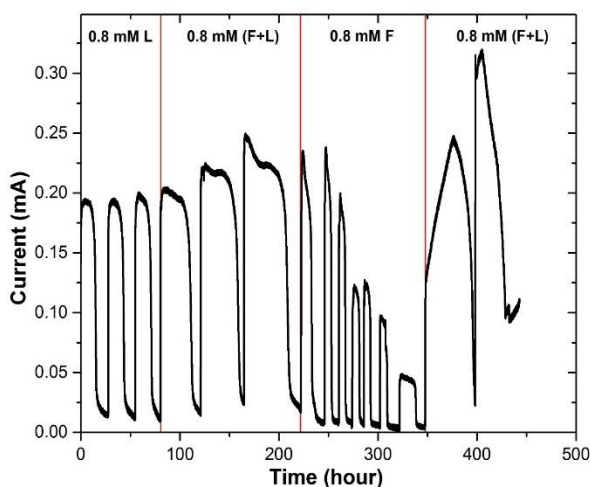


Figure 5.1 Current generation in the MFC supplied with various electron donors. Note: “L” means lactate; “F” means formate; “(F+L)” means addition of both substrate together; “0.8mM” means 0.8 mM of each substrate added each cycle.

Reproduced with permission from reference [211].

The peak current of the MFC was increased from 0.194 ± 0.005 mA using lactate alone to 0.231 ± 0.019 mA with additional formate as the extra electron donor ($p < 0.05$, one-tailed two-sample t-test with unequal variance at $\alpha = 0.05$ for all following statistical tests). Sole supply of formate significantly decreased the peak current to 0.147 ± 0.067 mA, lower than other conditions ($p < 0.05$). When extra lactate was added with formate at the last two cycles, the current was boosted up again, to 0.290 ± 0.056 mA, significantly higher than the current production with sole formate supply ($p < 0.05$). The catholyte pH was stable at 7.2-7.4 due to generally low current output and strong buffering capacity of phosphate buffer saline (PBS) [320], which eliminated the limiting effects of the cathode. The profile of current generation with the combination of formate and lactate is higher than that with only lactate or formate, indicating that formate and lactate could have achieved synergistic effect when metabolized by *S. oneidensis* MR-1 to benefit the electricity generation in the MFC.

5.3.2 Formate addition resulted in higher conversion efficiency

To further understand the impact of formate on the MFC performance containing *S. oneidensis* MR-1, CR (Coulombic recovery) and TC (total coulombs) are used to reflect the conversion

efficiency from electron donors to electrical output, with the equations below to calculate both indexes for each cycle (eqs. 5.1-3) [307-309]:

$$I = \frac{V}{R} \quad (5.1)$$

$$\text{TC of each cycle} = \sum I * t \quad (5.2)$$

$$\text{CR for consumption of formate and lactate} = \frac{\sum I * t * 1000}{(\Delta C_{\text{Formate}} * N_1 + \Delta C_{\text{Lactate}} * N_2) * V * F} \quad (5.3)$$

where in Eq. 1, V represents the voltage (V); R is the external resistance ($R = 8.2 \Omega$); where in Eq. 2, t represents the voltage measurement interval ($t=120$ s) of the digital multimeter; I represents the current (A) measured for each measurement interval, with the assumption that the current is the same within a time interval; and where in Eq. 3, $\Delta C_{\text{Formate}}$ and $\Delta C_{\text{Lactate}}$ (mM) represent the moles of formate and lactate added into the reactor at the beginning of each cycle, respectively; N_1 and N_2 represent the mole of electrons transferring per mole substrate consumption, respectively, where $N_1=2$ (formate $\rightarrow \text{CO}_2 + 2\text{H}^+ + 2\text{e}^-$) [321-323], and $N_2=4$ (lactate $+ 2\text{H}_2\text{O} \rightarrow \text{Acetate}^- + \text{HCO}_3^- + 5\text{H}^+ + 4\text{e}^-$, incomplete oxidation in anaerobic condition) [296, 310, 324, 325]; V represents the reactor volume (0.14L); F represents the Faraday constant (96485 Coulombs per mole electrons).

Combining non-labeled formate and lactate resulted in higher CR of $34.9 \pm 6.0 \%$ than that of lactate alone ($19.2 \pm 0.9 \%$) ($p<0.05$), indicating that extra formate addition on lactate supply can enhance the efficiency of substrate conversion to electrons, which can be ultimately harvested by the solid electron acceptor in the anode chamber (Figure 5.2). Supplying formate as the sole electron donor was found to have a lower CR of $19.3 \pm 6.8 \%$ ($p<0.05$) than that condition of combined formate and lactate, suggesting that lactate was also needed for *S. oneidensis* MR-1 to achieve good electron transferring efficiency. The TC with formate addition to lactate was 30.8 ± 5.4 C, much higher than the sum of TC (16.5 ± 2.1 C) via solely supplying formate (5.6 ± 1.9 C) and lactate (10.9 ± 0.2 C) ($p<0.05$) (Figure 5.2). These results demonstrate that the increased electricity generation with combined formate with lactate was not simply “1+1=2”; instead, the combination achieved “1+1>2”. This further confirms the pivotal role of formate in the electricity generation by *S. oneidensis* MR-1. Lactate is a commonly acknowledged an electron donor in MFCs, being involved in the central metabolism of *S. oneidensis* MR-1 [296, 302, 310, 315, 326],

while formate is more likely an electron transferring driving stimulus, which is related to the existence of FDH to convert the formate in periplasmic region to CO₂, and finally the electrons are released extracellularly [294, 306, 327]. Therefore, to explain the synergistic effects of lactate and formate in electricity generation in MFCs, we hypothesize that lactate may be more involved in central metabolism while formate may be more related to EET.

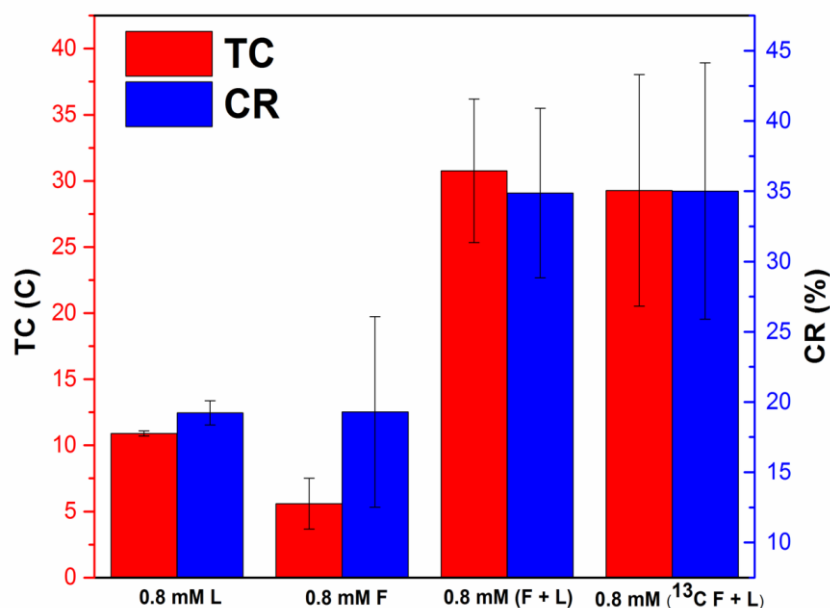


Figure 5.2 CR and TC obtained in the MFC under different supplies of formate and lactate. Note: “L” means lactate; “F” means formate; “(F+L)” means addition of both substrate together; “¹³C” represents the isotopomer addition; “0.8mM” means 0.8 mM of each substrate added each cycle.

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5.3.3 ¹³C tracer experiments facilitated the elucidation of the role for formate

To examine our hypothesis, we applied ¹³C tracer experiment to investigate the intracellular metabolism of *S. oneidensis* MR-1 when metabolizing formate and lactate in the MFC. The addition of ¹³C formate to non-labeled lactate also improved MFC electricity generation with the peak current of 0.308 ± 0.084 mA (Figure 5.3), suggesting that the labeled formate was also favored by *S. oneidensis* MR-1 to produce electricity. When the labeled formate was used with non-label lactate, similar improvements in both CR and TC were obtained (Figure 5.2). By examining the corrected mass distribution vectors from the isotopic analysis, no labeled carbon was found in the proteinogenesis amino acids (Table. 1), which are the building blocks for the cell

growth of both planktonic and biofilm cells in the MFC. This indicates that formate did not participate in the central metabolism to produce the building blocks for the cell growth. Instead, non-labeled lactate was mainly used for growth of the *S. oneidensis* MR-1. In other words, the cell growth and electricity generation could be decoupled when culturing *S. oneidensis* MR-1 with combined formate and lactate in MFCs. Indeed, it has been reported that lactate was a preferential carbon substrate than formate for cell growth of *S. oneidensis* MR-1. The biomass yield of *S. oneidensis* MR-1 cultivating under oxygen limited conditions reached >0.22 g/g when using lactate as the carbon substrate while only reached <0.11 g/g when using formate as the carbon substrate[328]. On the other hand, the expression levels of FDH genes in *S. oneidensis* MR-1 have been found to be up-regulated in MFCs [327]. Recently, a recombinant *S. oneidensis* MR-1 that harbored additional copies of FDH genes was found to generate electricity at a higher current density[294]. Therefore, it clearly suggested that the pivotal roles of formate played in supplying electrons through FDH in MFCs. Considering the importance of lactate and formate on cell growth and electricity generation, respectively, it is interesting but unsurprising to find in this study that these two carbon substrates could synergize with each other to improve current generation via a decoupled metabolism of cell growth and electricity generation.

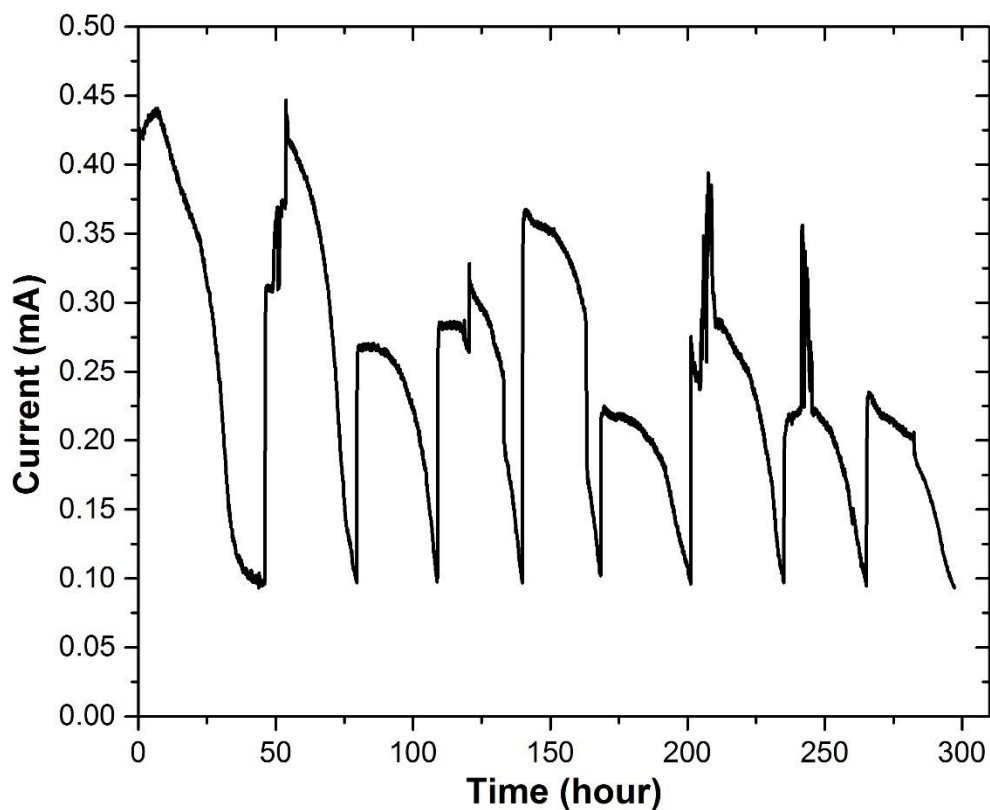


Figure 5.3 Current generation in the MFC supplied with 0.8 mM ^{13}C isotopic formate and 0.8mM non-labelled lactate.

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According to a proposed pathway shown in Figure 5.4, lactate is metabolized in the central metabolism of *S. oneidensis* MR-1 as its favorable substrate for cell growth [293, 294, 296, 302, 306, 310, 315, 326, 329], while formate is more likely used as an electron-transfer driving stimulus from either extracellular exchange or the central metabolism to release the electron in periplasm by FDH [294, 306, 327]. In this scheme, the unlabeled lactate would be directly metabolized via central metabolism to produce various unlabeled building block (e.g., unlabeled amino acids) as we observed, and the labeled formate would be transported into periplasm and oxidized by inner membrane FDH to CO_2 with the release of electrons, and eventually labeled carbon was lost in the form of CO_2 in the MFC system. The released electrons from formate would be further transported by EET to the anode electrode or riboflavin intermediate to enhance electricity generation. Therefore, such decoupled cell growth and electricity generation is responsible for the synergistic

improvement of electricity generation with the addition of formate during the lactate uptake by *S. oneidensis* MR-1 in MFCs.

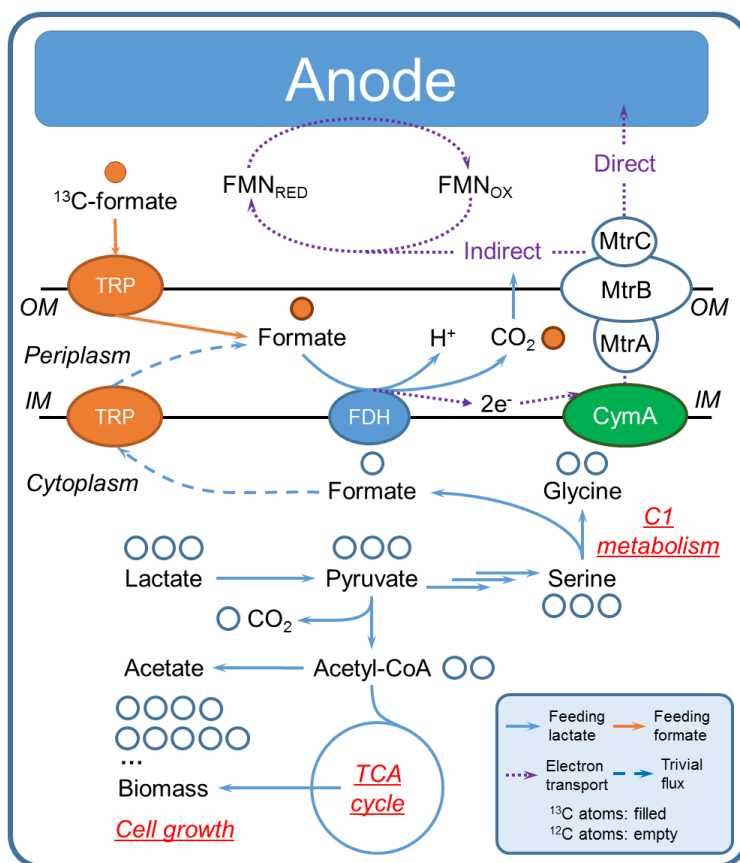


Figure 5.4 Proposed pathway of formate in the metabolism of *S. oneidensis* MR-1 after ^{13}C formate experimental analysis. Designation: IM: inner membrane; OM: outer membrane; FDH: formate dehydrogenase; FMN_{RED} : reduced flavin mononucleotide; FMN_{OX} : oxidized flavin mononucleotide ; CymA : tetraheme cytochrome anchored in inner membrane, accepts electrons from formate and transfers them to MtrA, MtrB and MtrC; MtrA, MtrB, MtrC: three kinds of periplasmic decaheme c-type cytochrome cytochromes anchored on outer membrane; TRP: formate transporter.

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5.3.4 Conclusions

In summary, this study has contributed to an initial understanding of the role of formate in the electricity generation by *S. oneidensis* MR-1 using lactate in MFCs. The results show that the co-supply of two substrates would affect the EET behaviors, and ultimately the electricity generation in the MFC, implying the conception of synergy in substrates. Mutually complementary substrates may take advantage of substrate interaction in the cell metabolism, and generate a total effect

greater than the sum of the individual contribution of single substrate for electricity generation. This may raise a question if other combinations of synergistic substrates also exist to enhance the MFC performance for pure or mixed culture. Moreover, ^{13}C tracer experiment proves to be an effective technique to explore the influence of substrates on EET behavior of a selected species. Future studies can target what substrate has greater stimulating effect on EET behavior qualitatively and quantitatively to potentially improve the electricity generation. It will not only help reveal the pathway of EET and carbon flow, but also formulate a strategy for adjusting substrate combination to achieve optimal electricity generation under a certain special condition (e.g., known composition of substrates being used for MFC application).

Chapter 6 A Multi-task Lasso Model for Investigating Multi-module Design Factors, Operational Factors and Covariates in Tubular Microbial Fuel Cells

(This section has been published as: Hongyue Sun, Shuai Luo (co-first author), Ran Jin and Zhen He. Multitask lasso model for investigating multimodule design factors, operational factors, and covariates in tubular microbial fuel cells." ACS Sustainable Chemistry & Engineering 3.12 (2015): 3231-3238.)

6.1 Introduction

Microbial fuel cells (MFCs) have been intensively studied in the past decade for energy recovery from waste and energy-efficient wastewater treatment [26]. By taking advantage of microbial interaction with solid electron acceptors/donors, electrons in organic compounds can be harvested as electrical energy through bio-electrochemical reactions [26]. Fundamental research has advanced our knowledge of mechanisms of electron transfer, materials for electrodes/separators, electrochemistry, microbiology, and system design/operation [330], which provides a foundation to evaluate MFC performance, optimize reactor design and scale up the system [114, 195, 288, 331-334].

Among the various MFCs that have been studied, tubular MFCs are of strong interest and have been investigated in great details through both laboratory and field operation for a long term (>450 days) [243, 250, 335, 336]. A tubular MFC has a configuration with low dead space, near plug-flow condition to allow sufficiently mixed flow regime, and high flexibility to connect additional tubular MFC for reactor extension [129, 131, 337]. Thus, the tubular MFC has great potential to be scaled up for energy recovery and wastewater treatment [129]. Recently, a 200-L tubular MFC system was reported for energy recovery from actual wastewater [338]. Despite great advancement and development of tubular MFCs, their performance of energy production and wastewater treatment is still limited by several key input variables, such as pH, hydraulic retention time (HRT), organic loading rate (OLR), temperature, and maintenance [27, 29, 249, 336, 339]. Those input variables have been studied experimentally but there is a lack of systematic approaches to investigate and understand their effects. To achieve that goal, mathematical modeling could be employed.

There have been several mathematical models developed for MFCs or related bioelectrochemical systems (BES) [115]. The initial MFC models, which are usually based on Monod equations, Faraday's Law and Nernst's equation etc., can predict the relationship between substrate consumption and electricity generation [115, 340]. Advanced models, such as two-dimensional models considering bulk liquid, electrode and biofilm in anode, are constructed to effectively predict the influence of multiple input variables (e.g., biofilm growth, pH, OLR) on MFC output variables [150, 341-344]. More details of MFC models can be found in a recent review paper [115]. These models have improved the understanding of the MFC energy recovery and waste treatment mechanisms; however, they usually predict one output variable at a time and do not perform systematic variable selection to identify the important input variables. For example, in many modeling efforts, the average output variables over a period of time are modeled, leading to multiple models together as a whole to predict the MFC performance. In addition, systematic variable selection has not been well performed in the modeling approach, which is very important to identify the subset of important input variables to predict and optimize the MFC performance. Therefore, a systematic modeling method is needed to quantitatively reveal the impact of various factors of anode (e.g., OLR, HRT) and cathode on the MFC performance.

A multi-task Lasso model (MLM) can be employed to model the MFC performance by multiple design factors, operational factors and covariates [345]. This is mainly because the MLM can predict multiple output variables with variable selection, and the model structure is explicit and interpretable. The Lasso model was proposed to identify significant input variables in a regression model with wide applications [346-348]. When an MFC system consists of multiple modules, the modeling of MFC performance measurement at certain module (e.g., from a certain time and module) is called one task. The "multi-task" in a MLM is to simultaneously model output variables at multiple modules [29]. In a MLM, groups of significant variables are also selected. This means that if an input variable is significant to predict an output variable at one-time instance, then the input variable will also be significant to predict the output at all-time instances. This is based on the engineering perception that the significance of input variables will remain unchanged over time, which allows better interpretations of the significance of the input variables. As a result, the penalty used in a MLM can identify significant variables over time for multiple output variables. This is extremely important when the sample size is limited, but there are many input variables at multiple

modules considered in the modeling step. It should be noted that other statistical models have also been employed for MFC modeling. For example, a relevance vector machine (RVM) was proposed for a single-chamber MFC modeling in the literature [349]. However, these models may not have an explicit model structure, or not be able to perform prediction in multiple tasks and variable selection [349].

In this study, a MLM was developed for modeling the performance of a tubular MFC that was divided into five modules (Figure 6.1), to identify the importance of the input variables. The cathode moisture, organic concentration, current, power density, NER (kWh/m^3 or kWh/kg COD) [29, 336], and organic removal efficiency can be obtained at each module. A full factorial design with four design factors (i.e., catholyte pump frequency, catholyte circulation rate, anolyte influent sodium acetate concentration, and anolyte flow rate) was performed. This is the first time that a MLM was employed to study the tubular MFC and the cathode moisture was included as an input variable. Cathode moisture is a unique feature for the present tubular MFC, which does not have the “cathode flooding” issue that is often an issue in hydrogen fuel cells.

6.2 Materials and Methods

6.2.1 MFC Setup and Operating

A tubular MFC was constructed using the carbon cloth coated with activated carbon as the cathode based on the previous study (Figure 6.1) [243], and more details about the MFC dimensions and setup can be found in the Appendix E. To study the dynamic changes of the key variables, the MFC was divided into five modules, which had separate cathode sharing the same anode (M1-M5, Figure 6.1). The anode compartment was inoculated with anaerobic sludge from Pepper’s Ferry Wastewater Treatment Plant (Radford, VA, USA). A synthetic organic solution with varied acetate concentration was continuously fed into the anode. In addition to acetate, this synthetic wastewater contained (per L of DI water): NH_4Cl , 0.15 g; NaCl , 0.5 g; MgSO_4 , 0.015 g; CaCl_2 , 0.02 g; NaHCO_3 , 0.1 g; KH_2PO_4 , 0.53 g; K_2HPO_4 , 1.07 g; and trace element, 1 mL [210]. The phosphate concentration used here was significantly higher than that in a municipal wastewater, mainly because of the need for anolyte buffer. The initial catholyte for each experimental run was composed of 1 L DI water containing 50 mL 100 mM phosphate buffered saline (PBS, 5.3 g/L KH_2PO_4 and 10.7 g/L K_2HPO_4) with the initial pH (~ 7.2) [350].

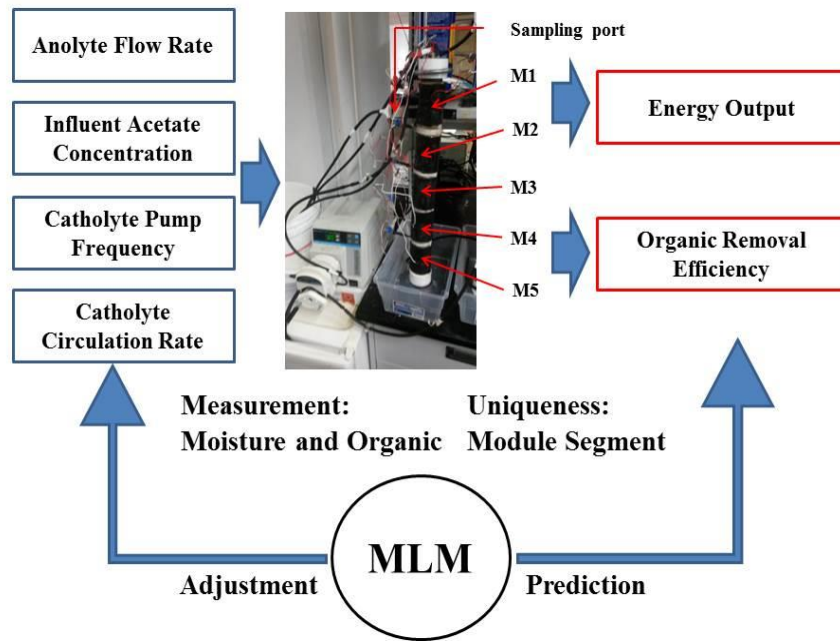


Figure 6.1 The flowchart of the model application in this study.

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6.2.2 Experimental Design and Measurements

A design of experiments (DOE) was used to collect data for understanding the impact of input variables on the MFC output variables [351]. A full factorial design was adopted here. There were four design factors (Table 6.1), and each factor was set to two levels. Because the curvature effect of these factors may not be significant based on the engineering perception, multiple levels were not used in this study. In total, there were 16 experimental runs (Table 6.2), and when a parameter was altered, the MFC system was operated for at least five days to reach a steady state before data collection, ensuring the repeatability and reliability of the data [352]. Each run was operated for three hours to collect the data (e.g., current and acetate concentration at each module). The model input variables include four design factors (x_1 - x_4), five-module moistures (x_5 - x_9) and five-module organic concentrations (x_{10} - x_{14}) as operational factors, and two covariates (i.e., catholyte pH and conductivity, x_{15} and x_{16}). The output variables include the five-module energy-related variables (i.e., current, power density, NER) and organic removal efficiency.

Table 6.1 Model input and output variables.

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Input variables	Design factors	x_1	Catholyte pump frequency (min/h)
		x_2	Catholyte circulation rate (mL/min)
		x_3	Anolyte influent sodium acetate concentration (mg/L)
		x_4	Anolyte flow rate (mL/min)
	Operational factors	x_5^a	Moisture at M1 (g)
		x_6	Moisture at M2 (g)
		x_7	Moisture at M3 (g)
		x_8	Moisture at M4 (g)
		x_9	Moisture at M5 (g)
		x_{10}^b	Organic at M1 (mg)
		x_{11}	Organic at M2 (mg)
		x_{12}	Organic at M3 (mg)
		x_{13}	Organic at M4 (mg)
		x_{14}	Organic at M5 (mg)
	Covariates	x_{15}	Catholyte pH
		x_{16}	Catholyte conductivity (mS/cm)
Output variables		Y_C^c	Current (mA)
		Y_{PD}	Power density (W/m ³)
		Y_{NERV}	NER (kWh/m ³)
		Y_{NERA}	NER (kWh/kg sodium acetate)
		Y_O	Organic removal efficiency (%)

a: moisture measurement at modules 1 to 5

b: organic concentration measurement at modules 1 to 5

c: $Y_C = (y_c^{(1)}, y_c^{(2)}, \dots, y_c^{(5)})^T$ is composed of the five-module output variables. $y_c^{(m)} = (y_{1,c}^{(m)}, y_{2,c}^{(m)}, \dots, y_{t,c}^{(m)})^T$ is the measurement from the m -th module, $m=1, 2, \dots, 5$ and $y_{t,c}^{(m)}$ represents the observation at the t -th time point and the m -th module, t is the number of

measurement points within a one-hour period. The same structure applies to Y_{PD} , Y_{NERV} and Y_{NERA} . Each of these Y_C , Y_{PD} , Y_{NERV} and Y_{NERA} is a response Y_i in Eq. (4).

Table 6.2 Experimental design matrix and experimental results.

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Run	Designed Values ^a				Experimental Results ^b		
	x_1^c	x_2	x_3	x_4	Power Density (W/m ³)	NER (kWh/m ³)	NER (kWh/kg sodium acetate)
1	60/0	50	500	2	6.190 ± 0.039	0.017 ± 0.001	0.192 ± 0.002
2	5/55	50	500	2	4.978 ± 0.365	0.013 ± 0.001	0.150 ± 0.011
3	60/0	20	500	2	5.807 ± 0.179	0.015 ± 0.001	0.176 ± 0.006
4	5/55	20	500	2	5.433 ± 0.303	0.014 ± 0.001	0.159 ± 0.009
5	60/0	20	250	2	3.299 ± 0.081	0.009 ± 0.001	0.173 ± 0.005
6	5/55	20	250	2	3.295 ± 0.211	0.009 ± 0.001	0.169 ± 0.011
7	60/0	50	250	2	2.889 ± 0.138	0.008 ± 0.001	0.155 ± 0.007
8	5/55	50	250	2	2.421 ± 0.182	0.006 ± 0.001	0.120 ± 0.007
9	60/0	20	250	3	3.630 ± 0.020	0.006 ± 0.001	0.249 ± 0.001
10	5/55	20	250	3	3.310 ± 0.237	0.006 ± 0.001	0.222 ± 0.015
11	60/0	50	250	3	3.712 ± 0.065	0.007 ± 0.001	0.274 ± 0.004
12	5/55	50	250	3	3.475 ± 0.247	0.006 ± 0.001	0.250 ± 0.016
13	60/0	20	500	3	3.925 ± 0.080	0.007 ± 0.001	0.138 ± 0.003
14	5/55	20	500	3	3.432 ± 0.201	0.006 ± 0.001	0.127 ± 0.008
15	60/0	50	500	3	4.381 ± 0.142	0.008 ± 0.001	0.149 ± 0.005
16	5/55	50	500	3	3.530 ± 0.353	0.006 ± 0.001	0.129 ± 0.012

a: x_1 : catholyte pump frequency (rinsing period (min) / stopped period (min)); x_2 : catholyte circulation rate (mL/min); x_3 : anolyte influent sodium acetate concentration (mg/L); x_4 : anolyte flow rate (mL/min)

Six anolyte measurements were taken from four sampling ports, the influent port and the effluent port every hour (i.e., at the end of 1st, 2nd and 3rd hour). The catholyte measurements were also conducted at the same locations and time. The measurements at these three-time instances were treated as different samples in the model. The data measurement details of voltage, pH, conductivity and acetate concentration can be found in the SI. To describe the output variables, power density (W/m³) based on the anode liquid volume, NER based on total anode flow rate (kWh/m³) and acetate loading rate (kWh/kg sodium acetate), and OLR were calculated according to previous studies [29, 336]. All equations for calculation are listed in the SI. Moisture of each cathode module was used to quantitatively represent the cathode environment (Figures E1 to E3).

6.2.3 Modeling Approach

Regression analysis is a powerful tool to study the relationship of the input variables and output variables [353]. The observed data are denoted as (X_i, y_i) , $i = 1, \dots, N$. The input variables $X =$

$(x_1, x_2, \dots, x_p)^T$ have p variables. A linear regression model is widely used to model the output variable y as:

$$y = \mathbf{X}^T \boldsymbol{\theta} + \varepsilon, \quad (6.1)$$

where $\boldsymbol{\theta} = (\theta_1, \theta_2, \dots, \theta_p)^T$ is the model coefficients vector, and ε is the residual term that follows independent and identically distributed normal distribution $N(0, \sigma^2)$. σ^2 is the variance of the residual. The model coefficient can be estimated by minimizing the mean squared error $\sum_i (y_i - \mathbf{X}_i^T \boldsymbol{\theta})^2$. However, Eq. (1) cannot be estimated when the number of input variables is large. In this study, there were only limited samples from DOE runs, while the model had many MFC design factors, operational factors and covariate as input variables. Thus, penalization was chosen to balance the trade-off between model complexity and prediction accuracy.

In general, different kinds of penalties can be used for model complexity penalization, such as L_0 , L_1 and $L_{1,2}$ penalties.[354] Therefore, the objective function for a model with penalization becomes:

$$\boldsymbol{\theta}(\lambda) = \min \sum_i (y_i - \mathbf{X}_i^T \boldsymbol{\theta})^2 + \lambda P(\boldsymbol{\theta}), \quad (6.2)$$

where $P(\boldsymbol{\theta})$ is the penalization function, and λ is a tuning parameter that controls the model training error and model complexity. Among the different penalizations, the Lasso model with L_1 penalty is with the form as follows [346]:

$$P(\boldsymbol{\theta}) = \|\boldsymbol{\theta}\|_1, \quad (6.3)$$

where $\|\boldsymbol{\theta}\|_1 = \sum |\theta_i|$ for a column vector $\boldsymbol{\theta}$; θ_i is the i -th element of $\boldsymbol{\theta}$. In recent years, the Lasso model is generalized to better handle problems with different data structures.[355, 356] Specifically, when there are multiple tasks, the multi-task Lasso model can be employed.[345] The MLM with $L_{1,2}$ penalty is shown in Eq. (6.4):[345]

$$\widehat{\boldsymbol{\beta}}(\lambda) = \arg \min \|\mathbf{Y} - \mathbf{X}\boldsymbol{\beta}\|_F^2 + \lambda \sum_{j=1}^p \|\boldsymbol{\beta}^{(j)}\|_2, \quad (6.4)$$

where $\mathbf{X} = (\mathbf{X}_1, \mathbf{X}_2, \dots, \mathbf{X}_N)^T$ is a $N \times p$ matrix; $\mathbf{X}_i$ is a $p \times 1$ vector representing the observation from the i -th sample, $i=1, 2, \dots, N$. The $p \times 1$ vector $\mathbf{X}_i = (x_{1,i}, x_{2,i}, \dots, x_{p,i})^T$ is composed of the input variables shown in Table 6.1. $\mathbf{Y} = (\mathbf{Y}_1, \mathbf{Y}_2, \dots, \mathbf{Y}_N)^T$ is a $N \times q$ matrix, and $\mathbf{Y}_i = (\mathbf{y}_i^{(1)}, \mathbf{y}_i^{(2)}, \dots, \mathbf{y}_i^{(5)})^T$ is a $q \times 1$ vector, which is composed of the five-module output variable measurements for the i -th sample. $\mathbf{y}_i^{(m)} = (y_{1,i}^{(m)}, y_{2,i}^{(m)}, \dots, y_{t,i}^{(m)})^T$ is the measurement from the m -

th module, $m=1, 2, \dots, 5$ and $y_{t,i}^{(m)}$ represents the observation at the t -th time instance and the m -th module in the i -th sample.

The data are collected every five minutes for energy-related output variables. In total, there are 12 data points at each of the five modules within a one-hour period. In total, there are 60 data points for energy-related output variables in a sample. Similarly, the organic removal efficiency is measured at the end of each hour and there is one data point at each of the five modules. In total, there are five data points for organic removal efficiency. We treat all these data points as multiple tasks in MLM modeling.

The notation and format for each output variable follows the same structure as $\mathbf{Y}_t$ (Table 6.1). $\boldsymbol{\beta} = (\boldsymbol{\beta}^{(1)}, \boldsymbol{\beta}^{(2)}, \dots, \boldsymbol{\beta}^{(p)})^T$ is a $p \times q$ matrix of the model coefficient. $\boldsymbol{\beta}^{(j)} = (\beta_1^{(j)}, \beta_2^{(j)}, \dots, \beta_q^{(j)})^T$ is the model coefficient vector for the multiple tasks at the j -th input variable, and $\beta_q^{(j)}$ is the q -th coefficient at the j -th input variable. These q model coefficients in $\boldsymbol{\beta}^{(j)}$ form a group, and are selected or eliminated simultaneously. Therefore, in the tubular MFC modeling, if a certain input variable is significant for one of the tasks (one of the 60 or 5 measurement points), then it will be important for other tasks. $\|\cdot\|_F$ is a Frobenius norm operator, i.e., for a $n \times p$ matrix A , $\|A\|_F = \sqrt{\sum_{i=1}^n \sum_{j=1}^p a_{i,j}^2}$, where $a_{i,j}$ is the element from the i -th row and j -th column of A . λ is a non-negative tuning parameter. If the tuning parameter λ goes to zero, the model is equivalent to the least squares estimation. If λ goes to a large number, the model will have limited number of significant predictors selected. The tuning parameter can be selected via Bayesian information criterion, cross validation (CV) or validation dataset [346].

To estimate the model coefficients $\boldsymbol{\beta}$, several optimization algorithms are proposed in the literature [345]. Specifically, the coordinate descent algorithm is shown to be a stable approach and can be easily scaled to large-scale problems. The basic idea of coordinate descent algorithm is to update each coordinate of model coefficients sequentially by fixing other coordinates. And this procedure is cycled over all the coordinates until convergence. For MLM, the algorithm is generalized to blockwise coordinate descent algorithm, where each $\boldsymbol{\beta}^{(j)}$ is treated as a block. The coefficients

within each block are updated simultaneously while fixing all other coordinates. More details of the algorithm can be found [345, 357].

6.3 Results and discussion

6.3.1 Cathode Moisture

Cathode moisture is a key factor for the cathode reaction of a tubular MFC with an air cathode (no cathode compartment). Moisture is introduced when the catholyte flows over the surface of the cathode electrode, and thus affected by both the catholyte flow/recirculation rate and circulation frequency (controlled by a pump). Figure E4 showed the moisture measurements for five modules under varied cathode operating conditions, and the cathode moisture increased from M1 to M5 with a higher circulation rate/pump frequency. The different moisture level among the modules was possibly related to the hydrophobicity of carbon cloth and water property [358]. The binding agent for catalysts coating - PTFE (Teflon) created stronger water repellence than the original carbon cloth and thus made the catholyte difficult to be retained on the cloth surface. On the other side, water viscosity and fluid friction helped water droplets to be retained on the cathode surface [359], and the water droplets were easier to accumulate at the downside (e.g. M4, M5) than the upside (e.g. M1, M2) because of gravity. The average moisture of M3 was unexpectedly lower than that of M2 under three operating conditions (i.e., 20mL/min 60/0, 50mL/min 5/55, 20mL/min 5/55) (Figure E4), probably due to the unevenness of activated carbon powder coated onto the carbon cloth, resulting in higher hydrophobicity to offset the effect of droplet accumulation on carbon surface.

6.3.2 Energy Recovery

Energy recovery is affected by both the cathode moisture and the anode organic input. Figure 6.2 shows the influence of varied operating conditions on NER (kWh/kg sodium acetate) of the entire tubular MFC, and Figure 6.3 shows the NER (kWh/kg sodium acetate) profile of each module from Run 13 to Run 16 under the same anode operation condition. The NER of Run 15 (0.149 ± 0.005 kWh/kg sodium acetate) was greater than that of Run 13 (0.138 ± 0.003 kWh/kg sodium acetate) ($p < 0.05$), indicating that a greater catholyte circulation rate significantly improved NER of the MFC (Figure 6.2A). In addition, under the same catholyte recirculation rate, increasing the catholyte recirculation frequency could also improve the NER (e.g., Run 13 > Run 14, and Run

15 > Run 16, with $p < 0.05$). Among the five modules of the MFC, the NER was also strongly related to the highest moisture of M5 producing the highest NER (Figure 6.3). Stopping the catholyte recirculation (e.g., in a one-hour period, Figure 6.3B and D) clearly decreased the NER. The higher catholyte pH of Runs 13 and 15 was the result of higher electricity generation and stronger cathode reaction (Figure E5) [360]. The reason why high moisture was accompanied with higher NER was related to better availability of conductivity electrolyte and promotion of proton diffusion to improve oxygen reduction and thus energy production [250, 361]. In addition, higher water content could improve local proton conductivity to transfer proton to cathode for facilitated cathode reaction [358].

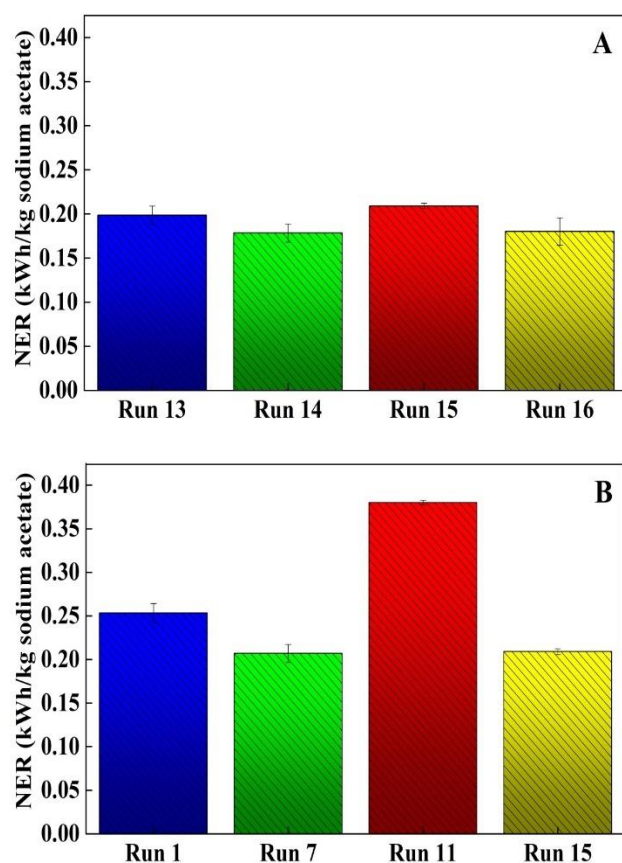


Figure 6.2 Impacts of varied operating conditions of the anode and the cathode on NER (kWh/kg sodium acetate) of the entire tubular MFC. (A) Same anode operating condition (500mg/L 3mL/min): Run 13: 20mL/min 60/0; Run 14: 20mL/min 5/55; Run 15: 50mL/min 60/0; Run 16: 50mL/min 5/55. (B) Same cathode operating condition (50mL/min 60/0): Run 1: 500mg/L 2mL/min (HRT: ~13 h); Run 7: 250mg/L 2mL/min (HRT: ~13 h); Run 11: 250mg/L 3mL/min (HRT: ~8 h); Run 15: 500mg/L 3mL/min (HRT: ~8 h).

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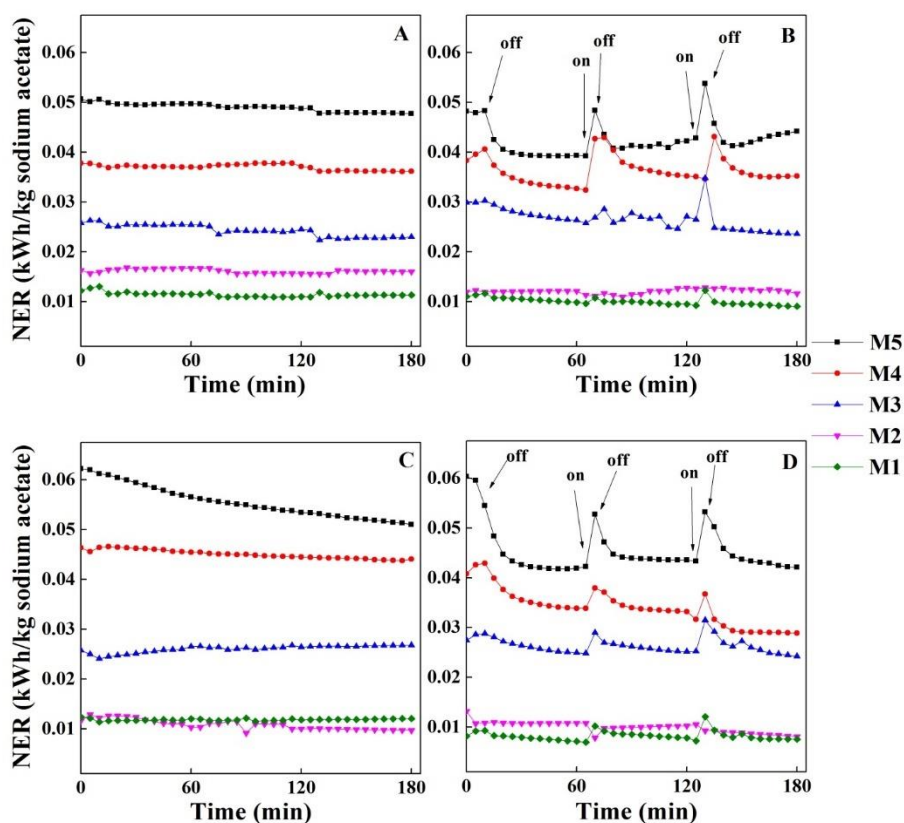


Figure 6.3 The NER (kWh/kg sodium acetate) of the modules (M1-M5) under the same anode operating conditions (500mg/L HRT of ~8hrs). (A) Run 13: 20mL/min 60/0; (B) Run 14: 20mL/min 5/55; (C) Run 15: 50mL/min 60/0; (D) Run 16: 50mL/min 5/55. Note: “on” means “turn on the catholyte pump”; “off” means “turn off the catholyte pump”.

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Under the same condition of the cathode moisture (50mL/min 60/0), increasing the OLR from 0.45 kg sodium acetate/(m³*d) (Run 7) to 0.67 kg sodium acetate/(m³*d) (Run 11) improved the NER from 0.155 ± 0.007 to 0.274 ± 0.004 kWh/kg sodium acetate, because of additional substrate supply at a higher OLR. However, further increase in OLR to 0.90 (Run 1) and 1.35 (Run 15) kg sodium acetate/(m³*d) decreased the NER to 0.192 ± 0.002 and 0.149 ± 0.005 kWh/kg sodium acetate, respectively (Figure 6.2B) [362], likely because OLR exceeded the conversion capacity of the electricigens inside the tubular MFC [336]. Figure E6 shows the acetate distribution pattern within the MFC anode from Run 13 to Run 16, indicating that the decreasing acetate concentration from M5 to M1 resulted in a decreasing NER trend (kWh/kg sodium acetate in Figure 6.3 and kWh/m³ in Figure E7) along M5 to M1 due to lower available substrate supply [131].

6.3.3 Organic Removal Efficiency

Experimental results show that the cathode moisture had a limited impact on organic removal efficiency, while the anode operating conditions could significantly influence the organic removal. Figures E6, E8 and E9 show acetate distribution through the anode under various operating conditions. Increased moisture has little positive influence on improving organic removal efficiency of the entire system (from Run 14 to Run 13, and Run 16 to Run 15 in Figures E6) and of each module (from Run 2 to Run 1, and Run 16 to Run 15 in Figure E8, and from Run 2 to Run 1, and Run 8 to Run 7 in Figure E9). In contrast, increasing OLR from 0.45 kg sodium acetate/(m³*d) (Run 7) to 0.9 kg sodium acetate/(m³*d) (Run 1), and then to 1.35 kg sodium acetate/(m³*d) (Run 15) reduced the organic removal efficiency from 88.2 ± 1.4% to 45.4 ± 0.9% with the same moisture, shown in Figure E10 [362]. A longer HRT could significantly increase organic removal efficiency from 45.4 ± 0.9 % (HRT 8.9 h of Run 15) to 80.1 ± 1.1 % (HRT 13.3 h of Run 1) because of more reaction time for acetate to be oxidized [336, 363].

6.3.4 Model Performance

The MLM proposed above is used for each output variables to illustrate the model performance. In total, there are 16 experimental runs, with 3-hour measurements at the end of each hour for each run. These measurement data are aligned and form the input (**X**) and output (**Y**) for the MLM model. The detailed format for the modeling data is shown in Table 6.1. The model coefficients are selected by tuning parameters λ via CV. To evaluate the MLM performance, 10-fold CV is adopted.[364] The basic idea of n -fold CV is to divide the data into n portions with equal or roughly equal sample size, and then use $(n-1)$ of the n portions to estimate the unknown parameters in the model (training step), and use the unused portion to evaluate the prediction performance (testing step). This training and testing steps will be repeated n times. In such a manner, the testing step prediction performance can be used to characterize the model performance. In the training step of each replication of the 10-fold CV, a 5-fold CV is used for the MLM tuning parameter λ (Eq. (1)) selection. Similar procedure can be found in a manufacturing system model [365].

To compare the model prediction performance in 10-fold CV, the root mean squared error (RMSE) was used (Eq. S14) [366]. The average model prediction error over 10-fold CV for current, power density, NER (kWh/m³), NER (kWh/kg sodium acetate) and organic removal efficiency are 4.12%,

5.02%, 4.42%, 9.38% and 3.59%, respectively, with details in Table E1. This demonstrates that the MLM can predict the tubular MFC with less than 10% error. For comparison, the separate multiple linear regression testing performance that is usually adopted in response surface method (RSM) is provided in Table E2. The corresponding average prediction errors are 43.61%, 58.71%, 56.59%, 178.73% and 28.99%. In addition, the 3rd one-hour period of Run 3 is used to illustrate the model prediction trend of current and organic removal efficiency (Figure 6.4). The prediction trends for other output variables are also illustrated (Figure E11). Based on the RMSEs and these figures, the MLM can adequately model the relationship among the input and output variables.

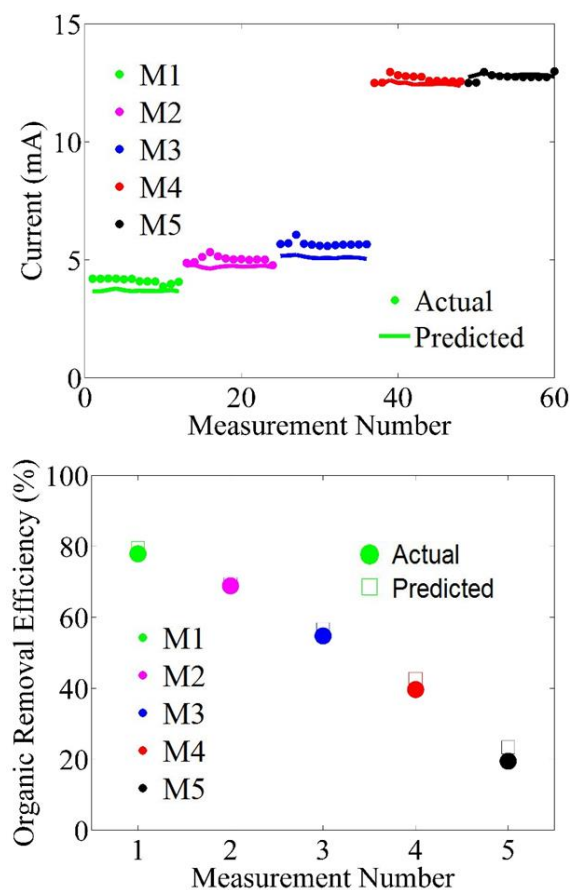


Figure 6.4 Experimental data (actual) versus modeled results (predicted): (A) current and (B) organic removal efficiency.

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Another important finding from the MLM model is the significance of the input variables, which is summarized in Figure 6.5. Each column represents an output variable, and each row represents the significance of an input variable. The color of each block represents the number of times that an input variable is selected in 10-fold CV. The variable significance can be identified if the

variable is selected more than five times over 10 replications in the 10-fold CV (Figure E12). In summary, four design factors, some operational factors and covariates are significant to predict the MFC output variables (Figure E12). It is interesting that the acetate concentration in M3 is less important for current and NER (kWh/m³) compared with other input variables, because of its moisture deviation and cathode material deviation mentioned above (Figure E4).

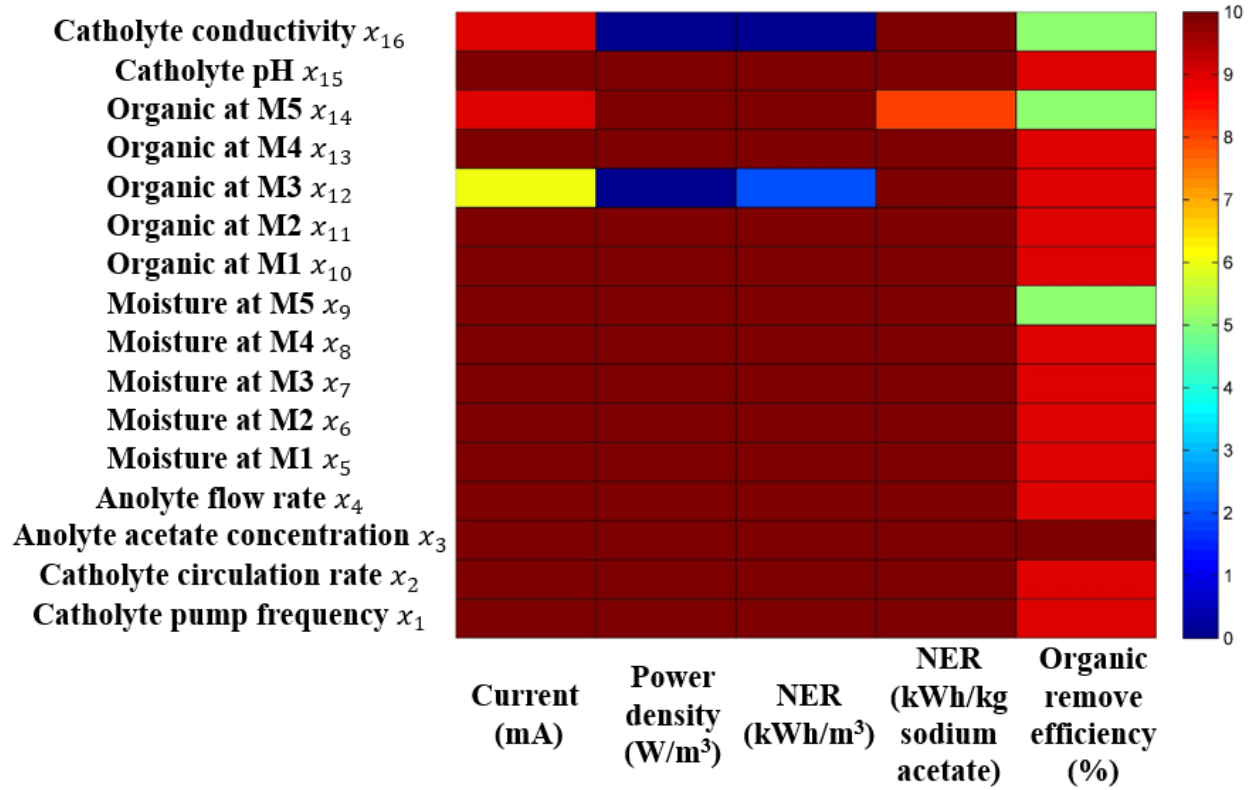


Figure 6.5 Significant variables for MLM (color represents number of times for a variable being selected over 10-fold CV).

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The model selection consistency for the MLM is also numerically evaluated. We randomly draw 70% of the data as training samples and the other 30% as testing samples, and repeated the modeling process for 1000 times. The corresponding variable selection results are provided in Figure E13 (where the color indicates the number of times that a variable is selected). If the number of selections in the model is close to 1000 or 0, the selection is consistent; if the number is around 500, the selection is not consistent. One can see that the model selection is consistent for most of the variables. Only a few variables (e.g., organic at M3 for current) are not consistent, which may be related to the spatially correlation among the variables from nearby modules. Such

inconsistence will be the subject of interest in the future work. It is also worth mentioning that the theoretical model selection consistency conditions for a Lasso model is provided. However, the consistency conditions for a multi-task Lasso are not available in the literature.

A quantitative relationship of variables is also identified in the MLM. Taking the NER (kWh/kg sodium acetate) of M5 at the end of each hour as an example, the model is shown as follows:

$$\hat{y} = -0.084710 + 0.000005x_1 + 0.000022x_2 - 0.000050x_3 + 0.053201x_4 + 0.002324x_5 + 0.003634x_6 - 0.004578x_7 + 0.000090x_8 + 0.001349x_9 - 0.044254x_{10} - 0.003918x_{11} + 0.001534x_{12} + 0.028547x_{13} + 0.000804x_{14} + 0.001249x_{15} - 0.005288x_{16}, \quad (6.5)$$

where x_1 to x_{16} represents the input variables (Table 6.1), and $\hat{y}$ is the predicted value for the NER (kWh/kg sodium acetate) of M5 at the end of each hour. Such a linear model is easy to be interpreted, and would provide an analytical form for tubular MFCs online monitoring and optimization. The residual analysis is used to validate the residual assumptions. These assumptions are not violated (the residual plots are provided in Figure E14).

6.3.5 Variable Importance

To further identify the relative importance of the input variables to the output variables, a linear regression is used to model the system overall performance (the summation of the energy-related output and organic removal efficiency over five modules), and to confirm the significant variables selected from the MLM. The p-values of the model coefficients are used to compare the relative importance of input variables (Table E3), and are calculated from the t-test to inference if an input variable is significant. The smaller the p-value, the more important an input variable is [367]. For energy-related output variables, p-values of anolyte influent flow rate, influent sodium acetate concentration and catholyte pump frequency are found to nearly zero, indicating that these input variables are very important for NER (Table E3). In contrast, the catholyte flow rate is not significant for NER. One possible reason is that the moisture increase caused by increasing catholyte flow rate (from 20mL/min to 50mL/min) in this study is lower than that caused by increasing the pump frequency (from 5/55 to 60/0) (Figure E4), which could reduce the relative significance of circulation rate on moisture change and further on NER. For the organic removal efficiency, the anode operating condition has more significant impact than the cathode operating condition (Table E3). The result is reasonable because moisture can only affect the proton

movement from anode to cathode, but have limited effect on acetate degradation due to membrane separation. Based on the observational data (Runs 1, 7, and 15 in Figure E10), proper control of anode is necessary to achieve effective organic removal.

6.3.6 Perspectives

This study has presented an MLM that is developed based on observational data and used to predict the output variables of a tubular MFC. It has demonstrated that the statistical model can serve as a powerful tool to quantitatively identify the effects of MFC input variables on output variables. The accurate prediction performance of the MLM indicates that the proposed model structure is capable of jointly predicting multiple tasks in output variables. Identification of the importance of the operational factors will be critical to further development of the tubular MFCs.

MLM could be further developed by considering those factors: first, current MLM mainly focuses on the process variables related to the moisture on the MFC performance, and many other parameters such as energy consumption by the pumps, initial pH and temperature, are also significant on MFC performance and will be studied in future work; second, anaerobic microorganisms on the anode, which include electrogenic and non-electrogenic bacteria, contribute to organic removal efficiency. The detailed values of these two groups should be considered to improve the model accuracy for the organic removal in future work; third, the use of acetate as a sole carbon source will not lead to good prediction for actual wastewater, and an ensemble modeling approach may be used to take advantages of the strength of both experimental data in lab-scale and observational data in real wastewater treatment [368]; fourth, the internal interactions of input variables were not considered in the MLM, because of their difficult interpretation (e.g., the interaction of moisture at M1 and M3 is hard to interpret) and future study will take the relationship of module-wise moisture and organic concentration into consideration via spatial models (in the spatial models, the interactions of the same type of variable at different modules (e.g., moisture at M1 and M3) can be better characterized and interpreted) [369, 370]; fifth, it is highly possible that the moisture reduction caused by reduced catholyte flow rate becomes roughly equal to that caused by the drying of cathode; and in such a case, the MFC performance could differ though the moisture reduction is the same; sixth, some output variables may have a nonlinear relationship with the input variables due to the complex system relationship, and advanced data

mining methods could be considered to approximate the nonlinear relationship by piecewise linear models [371]; and finally, monitoring and control method, on purpose of finding and solving the operational problems of the tubular MFC, can be developed based on the proposed MLM model [372].

Chapter 7 Ensemble modeling based on engineering and statistical models for parameter calibration towards smart design of microbial fuel cells

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7.1 Introduction

Recovering useful energy from wastewater is important to achieve environmental sustainability. Microbial fuel cells (MFCs) have become a promising treatment technology that uses microorganisms as biological catalysts to convert the organics in wastewater directly to electricity [24, 26]. Compared with the widely used anaerobic digester, MFCs can be operated at a relatively low temperature with less sludge production and lower energy demand [32]. Tremendous efforts have been made to improve MFC input factor settings for maximizing electricity generation and wastewater treatment efficiency [373]. A major challenge for MFC development is system scaling up [114], and there is a strong need for demonstrating the feasibility of this technology at a scale significantly larger than laboratory systems. At this moment, the design of MFCs is highly empirical, and the MFC performance is greatly affected by the difference in materials, design and operation. Enlarging MFC reactors lacks systematical guidance and optimization. Therefore, how to link bench scale MFCs to large system development and use the results obtained from bench studies to provide implications or guidance to design large scale MFCs becomes of strong interest. In this aspect, mathematical modeling could play a critical role.

Mathematical modeling can be categorized into engineering modeling and statistical modeling, according to different mathematical principles [161]. Engineering modeling for MFCs is based on the first principle governing the chemical and biological processes, which will remain unchanged under different conditions, except that some calibration parameters are sensitive to operation conditions [161]. Engineering modeling is capable of simulating MFC performance under various input factor settings [163, 166, 374]. However, due to the assumptions in modeling and numerical computation, engineering modeling may not accurately predict the MFC performance variables in real cases [145, 146, 161]. Moreover, many calibration parameters in engineering modeling need to be determined, and this will involve time-consuming experiments and computation for parameter calibration [145, 146]. Statistical modeling, on the other hand, can be built based on data set from MFCs [161, 246], and usually has higher accuracy when the prediction is made in

the neighborhood of the measurement data sets [146]. Some statistical models such as principal component analysis (PCA) is helpful to identify the major factors affecting MFC performance [375]. However, in extrapolation scenarios, the model estimated based on a data set from one input factor space is used for prediction at another input factor space, where input factor settings deviates from original input factor space of the training data set [246, 362, 376]. Such an extrapolation may be needed to explore a large input variable space to optimize the design of MFCs, but the extrapolation usually has a large prediction uncertainty (e.g., wide prediction intervals). To solve this problem, Bayesian methods are usually adopted to preserve information from historical measurement data sets, and update the model for extrapolation scenarios [168]. For an accurate model update, a large data set from the new scenarios will be required, but the large data set is usually hard to obtain.

Because engineering modeling can capture the first principles, while statistical modeling can reflect the actual measurements, we envision that the modeling prediction performance for extrapolation can be improved by integrating both the engineering model and data analytics into one model. An ensemble modeling approach is proposed for the model integration [184]. The “ensemble modeling” refers to modeling with different data sources, such as the simulation data from engineering model and measurement data from MFC system. In particular, a Gaussian Process (GP) model can serve as a surrogate model to represent the engineering model, and also be used to capture the discrepancy between the surrogate model and measurement data [185, 377, 378]. Such an approach will facilitate the engineering model parameter calibration and improve the model performance in extrapolation scenarios [185-187]. In addition, the number of samples required to construct an accurate model is significantly reduced compared with a statistical model which reaches to a comparable prediction accuracy. This is important as the fewer sample size will result in less cost and efforts in experiments for MFC design and optimization. The traditional GP models have been developed for a single output variable [185, 186], but are not capable of modeling a system with multiple output variables. Several works were performed to construct surrogate models based on engineering model [379, 380], but the joint modeling with an engineering model and measurement data was rare. To solve the problem, a framework was proposed for multiple output variables, and focused on model estimation and uncertainty analysis [378].

In this study, we newly proposed an ensemble modeling framework focusing on MFC extrapolation and design optimization. In particular, the effects of various sample sizes on the modeling performance were investigated under normal operation conditions of laboratory MFCs to reduce the efforts and cost during data collection for obtaining an accurate model. Rather than predicting at the original input factor space of the training data set [378], the ensemble model was evaluated for extrapolation. We further applied the ensemble model for MFC optimal design under extreme operation conditions that may provide guidelines for large system development.

7.2 Experimental and modeling

7.2.1 MFCs setup and operation

The experimental MFC system consisted of five tubular MFCs that were placed horizontally in serially hydraulic connection, which had actually working anode volume of ~200 mL / each MFC (diameter: 4cm, length: 15 cm), using synthetic wastewater to continuously and sequentially feed each MFC with details shown in Figure 7.1. The data of current generation and organic removal efficiency were collected from the entire MFC system (i.e., five MFCs were treated as “one MFC”). The anode electrode material was carbon brush (diameter: 2.6 cm, length: 11 cm), pretreated by the acetone and heated under 450 °C for 30 min, while cathode electrode was carbon cloth coated with activated carbon (5 mg cm⁻²), using 5% polytetrafluoroethylene (PTFE) as binder and the coated carbon cloth was heated at 370 °C for 30 min, prepared according to the previous works [209, 243, 381]. Anaerobic sludge from Peppers Ferry Wastewater Treatment Plant (Radford, VA, USA) was inoculated in the anodes. The synthetic wastewater contained (per L of deionized water): sodium acetate with varied concentration; NH₄Cl, 0.15 g; NaCl, 0.5 g; MgSO₄, 0.015 g; CaCl₂, 0.02 g; NaHCO₃, 0.1 g; KH₂PO₄, 0.53 g; K₂HPO₄, 1.07 g; and trace element, 1 mL [210]. The catholyte was composed of 4 L 2.5 mM phosphate buffered saline (PBS, 5.3 g L⁻¹ KH₂PO₄ and 10.7 g L⁻¹ K₂HPO₄) with initial pH at 7.2-7.4. The catholyte was recirculated to wet the cathode electrodes for oxygen reduction reaction (Figure 7.1). The external resistance for each MFC module was fixed at 2 Ω for high current generation.

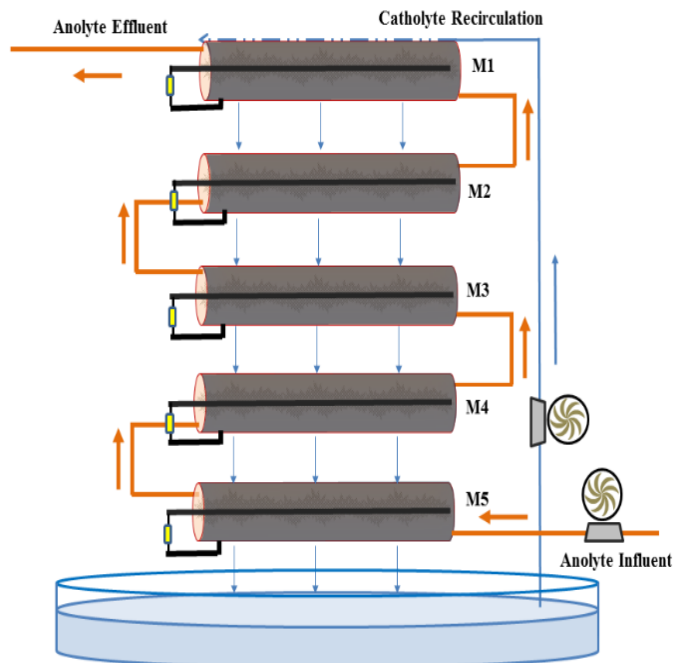


Figure 7.1 A schematic of the MFC system consisting of five tubular modules through hydraulic connection.

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7.2.2 Experimental design and measurements

To systematically investigate the impact of input factor settings on the MFCs performance, design of experiments (DOE) was used for data collection [351], and there were totally 56 experimental runs. Three input factors, anolyte flow rate (mL min^{-1}), initial acetate concentration (g L^{-1}), and catholyte pumping rate (mL min^{-1}) were considered (Table F1). Because the pH and conductivity of anolyte were kept at 6.8-7.1 and 5.7-6.0 mS cm^{-1} for all MFCs, while those numbers for catholyte stabilized around 7.1-7.4 and 0.7-0.9 mS cm^{-1} , the impact of other factors (e.g., pH, conductivity) on the model performance could be kept as constants at their optimized levels to eliminate their interference for the model construction and simulation [166]. In each experimental run, current generation, acetate concentration, cathode moisture were measured with the same procedures as the previous study [246]. In the DOE, normal and extreme operation conditions for the three input factors were explored with several sets of experiments, respectively. The objective is to evaluate the sample size effect and model extrapolation performance. In particular, we considered a normal operation region representing the operation condition similar to the normal treatment process on municipal wastewater (e.g., COD concentration at 0.20-0.45 g L^{-1} ,

approximately equivalent to 0.3-0.6 g L⁻¹ acetate in influent in this study) [32, 246, 362, 376], and two extreme operation regions representing operation conditions deviated from normal input factor settings to treat the municipal wastewater, which are considered as extrapolation scenarios. The experiments were carried out as follows: first, a full factorial design of 8 runs, and a space filling design of 32 runs were performed at normal operation region; then, two full factorial designs, each of 8 runs, were performed at extreme operation region 1 and 2, respectively (all settings listed in Table F1). Extreme operation region 2 extrapolated further away from normal operation region compared with extreme operation region 1 (e.g., acetate concentration at 1.5 and 4 g L⁻¹ for region 1, but more extreme at 1 and 5 g L⁻¹ for region 2). Each experimental run was operated for three hours and repeated for multiple times to assure model accuracy. The effluent sample of each MFC module was taken at the 1st hour and the 3rd hour (the data collected from each hour was treated as a sample in the modeling, and thus duplicate samples were obtained). Current interruption was used to measure internal resistance (Eq. (S1)) [383]. A GP model was applied to model the relationship between input factors and internal resistance (Table F2), and the GP model was applied to predict the internal resistance, which was applied as constant parameter to affect current generation in the ensemble model in this study. More details about internal resistance and GP model construction were explained in Appendix F.

7.2.3 Model formulation

The following assumptions were made during the model formulation: 1) anode and cathode overpotential in current calculation can be neglected due to sufficient buffer solution; 2) the microbial growth follows multiplicative Monod kinetics; 3) the error term in the ensemble model that captures the measurement uncertainty is assumed to be independent, identically and normally distributed; 4) the input factor settings in the optimal design can be in the original input factor space or extrapolation space. The detailed model formulations are described in the following sections.

7.2.3.1 Engineering model development

During the engineering model development, we considered anodic bacterial growth, substrate consumption, current generation, and internal resistance under the impact of various cathode moistures under certain assumptions (see details in Appendix F, Eqs. (F2)-(F22)) [150, 166]. Nine

parameters would need to be calibrated (i.e., calibration parameters) within their physical ranges (Table 7.1), based on the previous works [150, 166]. A surrogate model was constructed based on the simulation data to represent the engineering model. Therefore, this surrogate model can be used during the ensemble modeling to reduce the time-consuming engineering model execution. The simulation data were generated from a space filling design [384], in which 300 samples were generated with 3 input factors (all settings listed in Table F1), 9 calibration parameters (Table 7.1) and 2 output variables (i.e., current generation and effluent acetate concentration) to construct the ensemble model for the MFC system.

Table 7.1 Calibration parameters needed to be determined in the MFC engineering model. Reproduced with permission from reference [382].

Parameters	Ranges	Definitions
u_max_a	(0.1, 0.8)	Maximum anodophilic growth rate (day ⁻¹)
q_max_a	(1.5, 2.5)	Maximum anodophilic reaction rate (day ⁻¹)
K_s_a	(1, 50)	Half-rate constant of growth of anodophilic microorganisms (mg L ⁻¹)
k_m	(0.0500, 0.0501)	Mediator half-rate constant (dimensionless)
x_max_a	(50, 600)	Anodophilic biofilm space limitation (mg L ⁻¹)
x_max_m	(50, 600)	Methanogenic biofilm space limitation (mg L ⁻¹)
Y	(10, 20)	Estimated yield (dimensionless)
E_max	(0.6, 0.8)	Maximum cell potential (V)
M	(0.00015, 0.00030)	Total mediator fraction per unit microorganisms (dimensionless)

7.2.3.2. Ensemble modeling with GP model

The ensemble model was developed based on the entire MFC system. It was assumed that each MFC module in the MFC system was governed by the same underlying engineering model, and therefore, each MFC module had the same set of underlying calibration parameters. We denote $\mathbf{x}_i = (x_{1,i}, \dots, x_{p_1,i})^T$ as the MFC input factors in the i -th sample, and $\mathbf{t}_i = (t_{1,i}, \dots, t_{p_2,i})^T$ as the

engineering model calibration parameters in the i -th sample, and let $\mathbf{d}_i = (\mathbf{x}_i^T, \mathbf{t}_i^T)^T$. The true (unknown) values for the calibration parameters are $\boldsymbol{\theta} = (\theta_1, \dots, \theta_{p_2})^T$. The k output variables (e.g., current generation and organic removal efficiency for the MFC system) for the i -th sample from the simulation data are $\mathbf{y}_i^M = \boldsymbol{\eta}(\mathbf{x}_i, \mathbf{t}_i) = (y_{1,i}^M, \dots, y_{k,i}^M)^T$, and from the corresponding experimental measurement data are $\mathbf{y}_i^F = (y_{1,i}^F, \dots, y_{k,i}^F)^T$. We have $\mathbf{y}_i^F = \mathbf{y}_i^R + \mathbf{e}_i$, where $\mathbf{y}_i^R = \boldsymbol{\xi}(\mathbf{x}_i) = (y_{1,i}^R, \dots, y_{k,i}^R)^T$ is a vector of theoretical (unknown) output variables for the MFC system, and $\mathbf{e}_i \in R^{k \times 1}$ is a vector of errors caused by measurement. Here, the superscript “M” stands for the simulation data output variables, “R” stands for the theoretical output variables, and “F” stands for the measurement data output variables. The i -th sample $\mathbf{y}_i^F$ is modeled as [185, 378]:

$$\mathbf{y}_i^M = \boldsymbol{\eta}(\mathbf{d}_i) = \mathbf{m}(\mathbf{d}_i) + \boldsymbol{\varepsilon}(\mathbf{d}_i) \quad (7.1)$$

$$\mathbf{y}_i^R = \boldsymbol{\xi}(\mathbf{x}_i) = \boldsymbol{\eta}(\mathbf{x}_i, \boldsymbol{\theta}) + \boldsymbol{\delta}(\mathbf{x}_i) \quad (7.2)$$

$$\mathbf{y}_i^F = \mathbf{y}_i^R + \mathbf{e}_i = \boldsymbol{\eta}(\mathbf{x}_i, \boldsymbol{\theta}) + \boldsymbol{\delta}(\mathbf{x}_i) + \mathbf{e}_i = \mathbf{m}(\mathbf{x}_i, \boldsymbol{\theta}) + \boldsymbol{\varepsilon}(\mathbf{x}_i, \boldsymbol{\theta}) + \boldsymbol{\delta}(\mathbf{x}_i) + \mathbf{e}_i \quad (7.3)$$

Eq. (7.1) is the surrogate model for the MFC engineering model, where $\mathbf{m}(\mathbf{d}_i) = (m_1(\mathbf{d}_i), \dots, m_k(\mathbf{d}_i))^T$ and $\boldsymbol{\varepsilon}(\mathbf{d}_i)$ are the mean and covariance for the output variables at $\mathbf{d}_i$, respectively. We use a quadratic form to characterize the mean of output variables with MFC input factors and calibration parameters in the engineering model. $\mathbf{m}(\mathbf{d}_i) = (f(\mathbf{d}_i)^T \boldsymbol{\beta}_m)^T$, $f(\mathbf{d}_i) = (d_{1,i}, \dots, d_{p,i}, d_{1,i}^2, \dots, d_{p,i}^2)^T$, $p = p_1 + p_2$, where $\boldsymbol{\beta}_m = (\boldsymbol{\beta}_{m,1}, \dots, \boldsymbol{\beta}_{m,k})$ is the corresponding model coefficient matrix, and $\boldsymbol{\beta}_{m,k} \in R^{2p \times 1}$ is the coefficient vector for the k -th output variable. Eq. (7.2) captures the discrepancy of the engineering model output from the underlying MFC system output by the discrepancy term $\boldsymbol{\delta}(\mathbf{x}_i)$. $\boldsymbol{\eta}(\cdot)$ and $\boldsymbol{\delta}(\cdot)$ follow multivariate GP, respectively. Eq. (7.3) represents the relationship of actual measurement and theoretical (unknown) MFC output variables, where $\mathbf{e}_i \sim N\left(\mathbf{0}, \frac{1}{\lambda} \mathbf{I}\right)$ is the error term that is assumed to be independent, identically and normally distributed, λ controls the error precision and $\mathbf{I}$ is an identity matrix of dimension $k \times k$. Here the measurement data can be modeled by the decomposition of a surrogate model based on the MFC engineering model, a discrepancy term to capture the discrepancy of the surrogate model from real system, and an error term capturing the random measurement noise in Eq. (7.3). Therefore, the data generated from the engineering model and system measurement can be integrated via the ensemble model in Eqs. (7.1)-(7.3) for better MFC system modeling.

To characterize the covariance of $\boldsymbol{\eta}(\cdot)$ and $\boldsymbol{\delta}(\cdot)$, we use the squared exponential covariance function (Eqs. (F23) and (F24)) [378]. As a result, the output variables for all the simulation data follow $\mathbf{y}^M \sim N(\mathbf{m}^M, \Sigma^M)$, and the output variables for all the measurement data follow $\mathbf{y}^F \sim N(\mathbf{m}^F, \Sigma^F)$, $\Sigma^F = \Sigma^{\eta,F} + \Sigma^\delta + \Sigma^e$, where $\mathbf{y}^M = (y_{1,1}^M; \dots; y_{1,m}^M; \dots; y_{k,1}^M; \dots; y_{k,m}^M)$ and $\mathbf{y}^F = (y_{1,1}^F; \dots; y_{1,n}^F; \dots; y_{k,1}^F; \dots; y_{k,n}^F)$. “;” stands for the column-wise concatenation, m is the number of samples in the simulation data, and n is the number of samples in the measurement data. In the above notation, $\mathbf{m}^M$ and $\mathbf{m}^F$ is the mean for $\boldsymbol{\eta}(\cdot)$ at all the simulation data and measurement data, respectively. Σ^M and $\Sigma^{\eta,F}$ is the covariance for $\boldsymbol{\eta}(\cdot)$ at all the simulation data and measurement data, respectively. Σ^δ and Σ^e is the covariance for discrepancy and error term at all the measurement samples, respectively (see details in the Appendix F). Denote $\mathcal{D} = (\mathbf{y}^F; \mathbf{y}^M)$ as all the measurement data and simulation data collected from the MFC. The likelihood function for the data,

$$L(\mathcal{D}|\boldsymbol{\theta}, \boldsymbol{\beta}_m, \emptyset_j, \Sigma^e, j = 1, \dots, k) \propto |\Sigma|^{-1/2} \exp\left\{-\frac{1}{2}(\mathcal{D} - \boldsymbol{\mu})^T \Sigma^{-1}(\mathcal{D} - \boldsymbol{\mu})\right\}$$

where $\boldsymbol{\mu} = (\mathbf{m}^F; \mathbf{m}^M)$ is the mean for $\mathcal{D}$, and $\Sigma = \begin{bmatrix} \Sigma^F & \Sigma^{F,M} \\ \Sigma^{M,F} & \Sigma^M \end{bmatrix}$ is the corresponding covariance matrix, $\Sigma^{M,F}$ is the cross-covariance among the simulation and measurement data, and $\emptyset_j$ is a set of parameters for the covariance matrix (see details in the Appendix F). The ensemble model estimation is performed under Bayesian framework, where the prior specifications of the parameter distributions are adjusted by the measurement data and simulation data [185, 378]. As a result, the posterior distribution of the parameters can be calculated based on the prior and the likelihood with the form,

$$\pi(\boldsymbol{\theta}, \boldsymbol{\beta}_m, \emptyset_j, \Sigma^e, j = 1, \dots, k|\mathcal{D}) \propto L(\mathcal{D}|\boldsymbol{\theta}, \boldsymbol{\beta}_m, \emptyset_j, \Sigma^e, j = 1, \dots, k) \times \pi(\boldsymbol{\theta}) \times \prod_{j=1}^k (\pi(\emptyset_j))$$

where $\pi(\cdot)$ denotes the prior distribution. We followed a previous work for the specifications of prior distributions [378]. The parameter estimation was performed using Monte Carlo Markov Chain (MCMC), see details in [378, 385]. Σ^e was estimated from the measurement data, and $\boldsymbol{\beta}_m$ was estimated via ridge regression at each MCMC iteration [364].

7.2.3.3. MFC design optimization

The ensemble model constructed from Section 7.2.3.2 was used for optimized MFCs design in new MFC conditions. The design can provide guidance for the optimization of MFC

configurations such as chamber dimension and membrane type, or the optimization of MFC operation factors. Here, we focus on the latter design to optimize the MFC input factor settings for improving current generation and organic removal efficiency, while a similar approach can be used for optimizing the MFC configurations. The advantage of such a design is that the time-consuming trial-and-error input factor setting explorations for new conditions can be eliminated. In the design, the criterion is to yield high electricity generation and organic removal efficiency (“larger-the-better” criterion) [351]. Given the known observation $X = (\mathbf{x}_1^T; \dots; \mathbf{x}_n^T)$ and the corresponding output variables $\mathbf{y}$, the output variables $\mathbf{y}^*$ of any set of new X^* are predicted with [386],

$$\mathbf{y}^* \sim N(\boldsymbol{\mu}_{\mathbf{y}^*}, \Sigma_{\mathbf{y}^*}) \quad (7.4)$$

$$\boldsymbol{\mu}_{\mathbf{y}^*} = \boldsymbol{\mu}_{\mathbf{y}} + \Sigma_{X^*,X} \Sigma_{X,X}^{-1} (\mathbf{y} - \boldsymbol{\mu}_{\mathbf{y}}), \Sigma_{\mathbf{y}^*} = \Sigma_{X^*,X^*} - \Sigma_{X^*,X} \Sigma_{X,X}^{-1} \Sigma_{X,X^*}$$

where $\boldsymbol{\mu}_{\mathbf{y}^*}$ and $\Sigma_{\mathbf{y}^*}$ are mean and covariance of $\mathbf{y}^*$. $\boldsymbol{\mu}_{\mathbf{y}}$ is mean for $\mathbf{y}$, $\Sigma_{X,X}$ is covariance among the samples in X , Σ_{X^*,X^*} is covariance among the samples in X^* , and Σ_{X,X^*} is covariance across the samples in X and X^* . The covariance can be obtained by applying Eqs. (F23) and (F24) to X and X^* with calibration parameters fixed at the calibrated values. To balance the trade-off between current generation and organic removal efficiency, a weighted objective function is used with a weight factor w , as shown in Eq. (7.5). We rescale the current generation and organic removal efficiency values to the range of [0, 1] so that they are comparable in terms of scales. The design objective function is,

$$\min - (w y_{curr}^*(\mathbf{x})^2 + (1 - w) y_{orga}^*(\mathbf{x})^2) \quad (7.5)$$

subject to $l_i \leq x_i \leq u_i, i = 1, 2, 3$

where $\mathbf{x} = (x_1, x_2, x_3)^T$ is a vector of input factors, i.e., anolyte flow rate (x_1), influent acetate concentration (x_2) and catholyte pump rate (x_3). $y_{curr}^*(\cdot)$ and $y_{orga}^*(\cdot)$ are the predicted (rescaled) current generation and organic removal efficiency values. The constraints l_i and u_i are specified as the lower bound and upper bound of factors in the extreme operation regions (Table F1).

7.3. Results and discussion

7.3.1. MFCs performance

The current generation of the MFC system maintained at ~30 to 40 mA under the normal operation region, while the current fluctuated under two extreme operation regions (Figure F1A, in Appendix F, and similar definition for “S” thereafter). A low catholyte flow rate (e.g., 1.0 to 1.5 mL min⁻¹)

strongly inhibited current generation because of insufficient water content on the cathode surface that will negatively affect ion transport across cation exchange membrane and supplying protons to oxygen reduction reactions. However, an extremely high catholyte flow rate (e.g., above 75 mL min⁻¹) led to liquid flushing on the cathode material and flooding phenomena with no three-phase interface, reducing the contacting surface between proton and oxygen to finally impede reduction reaction and decreasing the current generation. In this study, higher organic loading rate (especially in a space filling design from run 9 to run 40) generally resulted in lower organic removal efficiency (Figure F1B), confirming the result of a previous work [246]. In conclusion, the MFC system was functional and responsive to variation of the operational factors.

7.3.2. Ensemble model evaluation

The ensemble model was compared with a statistical model built from the measurement data. In the statistical model, an l_1 penalized linear regression (i.e., a Lasso regression model [246, 346], see details in Appendix F) was used for modeling current generation and organic removal efficiency, respectively (Eq. (F25)). Moreover, to provide practitioners a guideline for model construction, the sample size was varied during model construction and evaluation to be 10%, 30%, 50%, 70% and 90% of all the samples collected in the normal operation region (the corresponding models were denoted as Normal_Region_10%, Normal_Region_30%, Normal_Region_50%, Normal_Region_70%, and Normal_Region_90%, respectively). Under each sample size, we evaluated the model prediction performance with 10-fold Cross Validation (CV) [364]. Average normalized root mean squared errors (NRMSEs) over the 10-fold CV are provided in Table 7.2, where NRMSE was defined in the previous work [246]. The smaller the NRMSE is, the better the model performs. The ensemble model had smaller average NRMSE than the statistical model for both current generation and organic removal efficiency (Table 7.2), especially when the sample size was small. For example, for current generation, the NRMSE of the proposed ensemble model at Normal_Region_10% was 7.80, which is smaller than that of the Lasso model (i.e., 17.18). For organic removal efficiency, the NRMSE of the proposed ensemble model is 5.42, which is also smaller than that of the Lasso model (i.e., 10.70). As an illustration, the predicted values from the ensemble model and the statistical model under Normal_Region_90%, along with measurement data for both current generation and organic removal efficiency, are shown in Figure 7.2A and 7.2B, respectively. The ensemble model had better prediction output than the statistical model,

because it integrated the engineering model and data analytics. The ensemble model required fewer measurement data to achieve the comparable modeling accuracy to that of the statistical model under the normal operation region. This is a desirable property because fewer experimental runs are needed for MFC modeling to achieve high prediction accuracy.

There are limited experimental resources in the extrapolation input factor space. The statistical model may have poor prediction performance for extrapolation, but an ensemble model may alleviate this problem [145, 364]. The ensemble model and the statistical model were further compared for the prediction in extrapolation scenarios. In these models (denoted as Extrapolation_Region), all the data from normal operation region were used as training data, and the data in extreme operation regions (extreme operation region 1 and extreme operation region 2) were used for model evaluation (i.e., testing data). NRMSEs were used for model comparison (Table 7.3). The ensemble model performed much better (much smaller average NRMSE) than the statistical model for both current generation and organic removal efficiency. For example, the NRMSE at Extreme Region 1 was 10.92 in the ensemble model and 21.06 in the Lasso model for current generation, and 11.56 in the ensemble model and 28.38 in the Lasso model for organic removal efficiency. The predicted values from the ensemble model and the statistical model under Extrapolation_Region along with measurement data for both current generation and organic removal efficiency are shown in Figure 7.2C and 7.2D, respectively. The results indicated that the predicted values from the ensemble model have consistent patterns with the measurement data. In addition to model prediction comparison, the model assumptions are validated. A representative residual plot is shown in Figure 7.3A (more residual plots for Normal_Region_90% and Extrapolation_Region are available in Figures F2-F3). The assumptions in the ensemble model and statistical model that the residual followed independent and identically $N(0, \sigma^2)$ distribution was not violated (Figures 7.3A, F2-F3), indicating the ensemble model worked to fit the measurement data.

Table 7.2 Comparison of average NRMSE (%) for ensemble (GP) model and statistical model for normal operation region over different training sample sizes.

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	Curr_GP		Curr_Stat		Orga_GP		Orga_Stat	
	Tr.	Te.	Tr.	Te.	Tr.	Te.	Tr.	Te.

Normal_Region_10%	0.96 (0.37)	7.80 (0.22)	0.21 (0.03)	17.18 (1.64)	0.60 (0.11)	5.42 (0.16)	0.48 (0.11)	10.70 (0.61)
Normal_Region_30%	1.24 (0.15)	5.91 (0.17)	2.28 (0.24)	12.12 (0.56)	0.64 (0.08)	4.12 (0.11)	1.58 (0.15)	5.29 (0.10)
Normal_Region_50%	1.36 (0.09)	5.45 (0.28)	3.90 (0.23)	6.65 (0.29)	0.70 (0.11)	3.30 (0.52)	1.75 (0.03)	4.29 (0.08)
Normal_Region_70%	1.91 (0.11)	4.94 (0.51)	4.40 (0.09)	6.07 (0.16)	1.06 (0.03)	3.40 (0.11)	1.93 (0.04)	4.77 (0.20)
Normal_Region_90%	1.49 (0.07)	2.97 (0.26)	4.77 (0.03)	4.95 (0.62)	1.13 (0.02)	3.33 (0.64)	2.41 (0.02)	2.85 (0.51)

Note: “Curr_GP” and “Curr_Stat” represent average NRMSE for current generation in ensemble (GP) model and statistical model respectively; “Orga_GP” and “Orga_Stat” represent average NRMSE for organic removal efficiency in ensemble (GP) model and statistical model respectively; “Tr.” and “Te.” represent training and testing data; values in the parenthesis represent the standard deviation of the NRMSE over 10 CVs.

Table 7.3 Comparison of NRMSE (%) for ensemble (GP) model and statistical model for two extreme operation regions.

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	Curr_GP	Curr_Stat	Orga_GP	Orga_Stat
Training	1.19	4.61	1.09	2.43
Extreme Region 1	10.92	21.06	11.56	28.38
Extreme Region 2	23.88	43.61	20.98	61.47
Extreme Region Overall	15.94	29.50	16.74	47.44

Note: “Curr_GP” and “Curr_Stat” represent average NRMSE for current generation in ensemble (GP) model and statistical model respectively; “Orga_GP” and “Orga_Stat” represent average NRMSE for organic removal efficiency in ensemble (GP) model and statistical model, respectively.

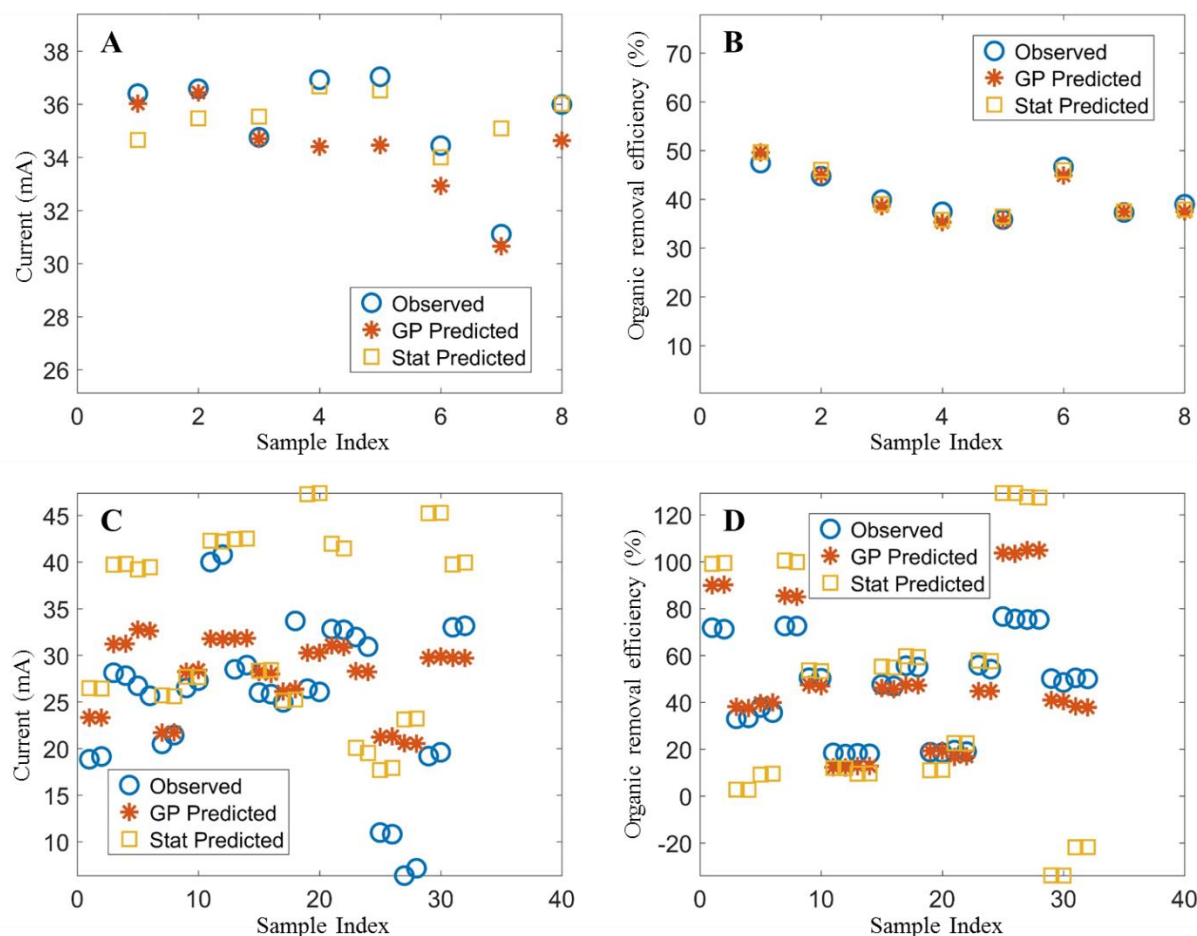


Figure 7.2 Comparison of testing data (Observed), ensemble (GP) model predicted value (GP Predicted) and statistical model predicted value (Stat Predicted): (A) current generation and (B) organic removal efficiency prediction with Normal_Region_90%; (C) current generation and (D) organic removal efficiency prediction with Extrapolation_Region. Note: the number of testing samples in Normal_Region_90% is 8 (A and B), and the number of testing samples in Extrapolation_Region is 32 (C and D).

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The parameter calibration performance was also evaluated. A representative calibration parameters' posterior distribution plot is shown in Figure 7.3B (more plots are shown in Figures F4-F5 in the Appendix F). The calibration parameters generally had a peak probability density function value from the MCMC evaluations (Figures 7.3B, Figures F4-F5), where the peak corresponded to the value that a random variable (i.e., a calibration parameter) had the highest probability to take. Therefore, the peak value reflected the calibrated value for the calibration parameters. As a result, the ensemble model could help identify the calibration parameter values in the engineering model. The posterior median values of the calibration parameters for Normal_Region_90% and

Extrapolation_Region are shown in Table 7.4. Due to the difference in the training data set and the irregular response surface (Figure 7.4, where a global optimum was not guaranteed for the response surface), the calibration parameter values can be quite different for these two models. The response surfaces of the ensemble model for current generation and organic removal efficiency are provided in Figure 7.4, from which one can observe that the response surfaces were highly nonlinear, where the solution was easily trapped into local optimum [387]. Another possible reason for the difference in the calibration parameter values of these two models is that some of the calibration parameters (such as K_{s_a} , x_{max_m} and Y) are not sensitive. The changes in the values of these insensitive calibration parameters would not affect the optimization much, and their values during the ensemble model parameter estimation can be different in the two models.

Table 7.4 Calibrated calibration parameters in ensemble (GP) model under Normal_Region_90% and Extrapolation_Region.

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Parameters	Normal_Region_90%	Extrapolation_Region
u_{max_a} (day ⁻¹)	7.63×10^{-1}	6.86×10^{-1}
q_{max_a} (mg day ⁻¹)	2.48	1.62
K_{s_a} (mg L ⁻¹)	4.84×10^1	8.41
k_m (dimensionless)	5.01×10^{-2}	5.00×10^{-2}
x_{max_a} (mg L ⁻¹)	2.43×10^2	5.98×10^2
x_{max_m} (mg L ⁻¹)	3.74×10^2	4.51×10^2
Y (dimensionless)	1.06×10^1	1.97×10^1
E_{max} (V)	7.83×10^{-1}	6.24×10^{-1}
M (dimensionless)	2.32×10^{-4}	1.85×10^{-4}

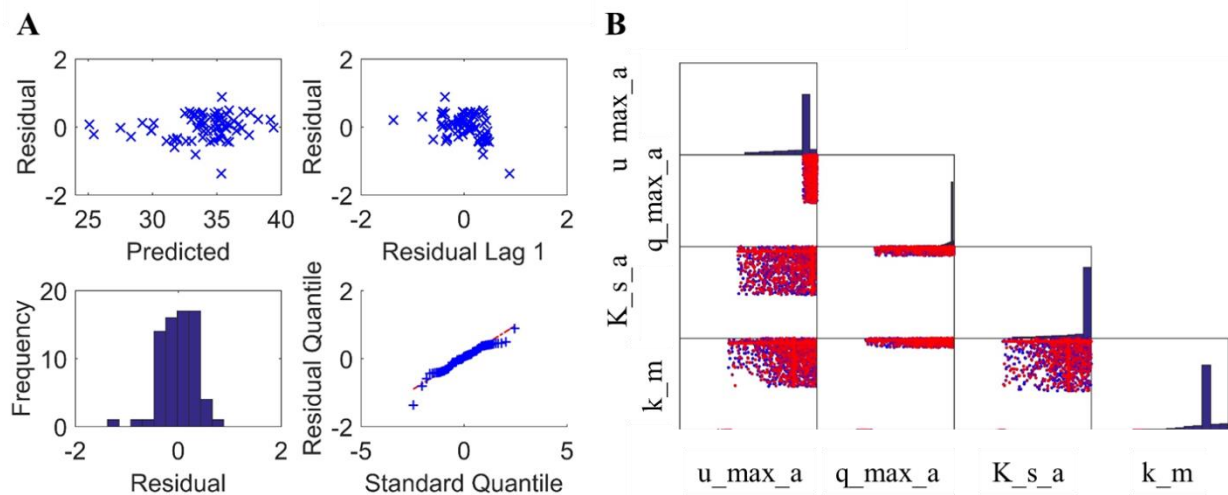


Figure 7.3 Representative residual plot and calibration parameter posterior distribution plot: (A) residual plot for current generation in ensemble (GP) model under Normal_Region_90%, and (B) posterior distribution of the first four calibration parameters under Normal_Region_90%. Note: each diagonal plot in (B) shows the histogram of posterior samples drawn from the posterior distribution of each calibration parameter during MCMC execution, and each off diagonal plot in (B) shows the scatter plot of posterior samples drawn from the pairwise joint posterior distribution of the corresponding calibration parameters during MCMC execution (see detailed explanations in Appendix F).

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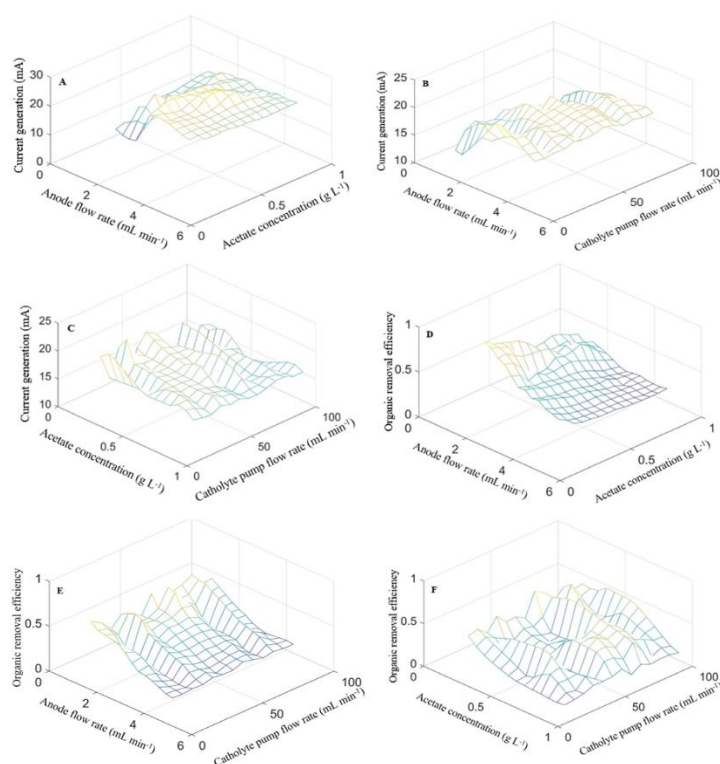


Figure 7.4 Response surface of ensemble (GP) model for current generation and organic removal efficiency under various combination of input factors: current generation (mA) under input factors of (A) anode flow rate/acetate concentration, (B) anode flow rate/catholyte pump flow rate, and (C) acetate concentration/catholyte pump flow rate; organic removal efficiency under input factors of (D) anode flow rate/acetate concentration, (E) anode flow rate/catholyte pump flow rate, and (F) acetate concentration/catholyte pump flow rate. In each figure, the vertical axis represents the current generation or organic removal efficiency, and the horizontal axis represents the input factors.

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7.3.3. Sensitivity analysis for the ensemble model

Each calibration parameter was changed by -100%, -50%, 0%, 50% and 100% from the calibrated parameter value, and the corresponding model prediction NRMSEs were summarized. As an illustration, the sensitivity analysis results of calibrated parameters under Normal_Region_90% is provided in Figure 7.5 (see Figure F6 for the sensitivity analysis under Extrapolation_Region). By changing the same percentage of the calibrated parameter value, a larger change in NRMSE indicated that the targeted calibration parameter was more sensitive to impact the output variable in this study. Based on the current generation in Normal_Region_90%, the parameters such as maximum specific growth rate (u_{max_a}), half-rate constant of anodophilic bacteria (K_{s_a}), maximum substrate degradation rate (q_{max_a}), maximum attainable concentrations for anodophilic and methanogenic bacteria (x_{max_a} and x_{max_m}), and total medium fraction (M) had significant impact on the model output variable. These parameters are significant because they are related to the activity and growth of anodophilic bacteria, and electron transportation from anodophilic bacteria to the electrode for current generation [166]. Based on organic removal efficiency, q_{max_a} and x_{max_a} also had significant impacts, because these parameters are relevant to the organic degradation efficiency of anodophilic bacteria in the anode, which affects the organic removal efficiency. Thus, adjustment of q_{max_a} and x_{max_a} would significantly impact both current generation and organic removal efficiency.

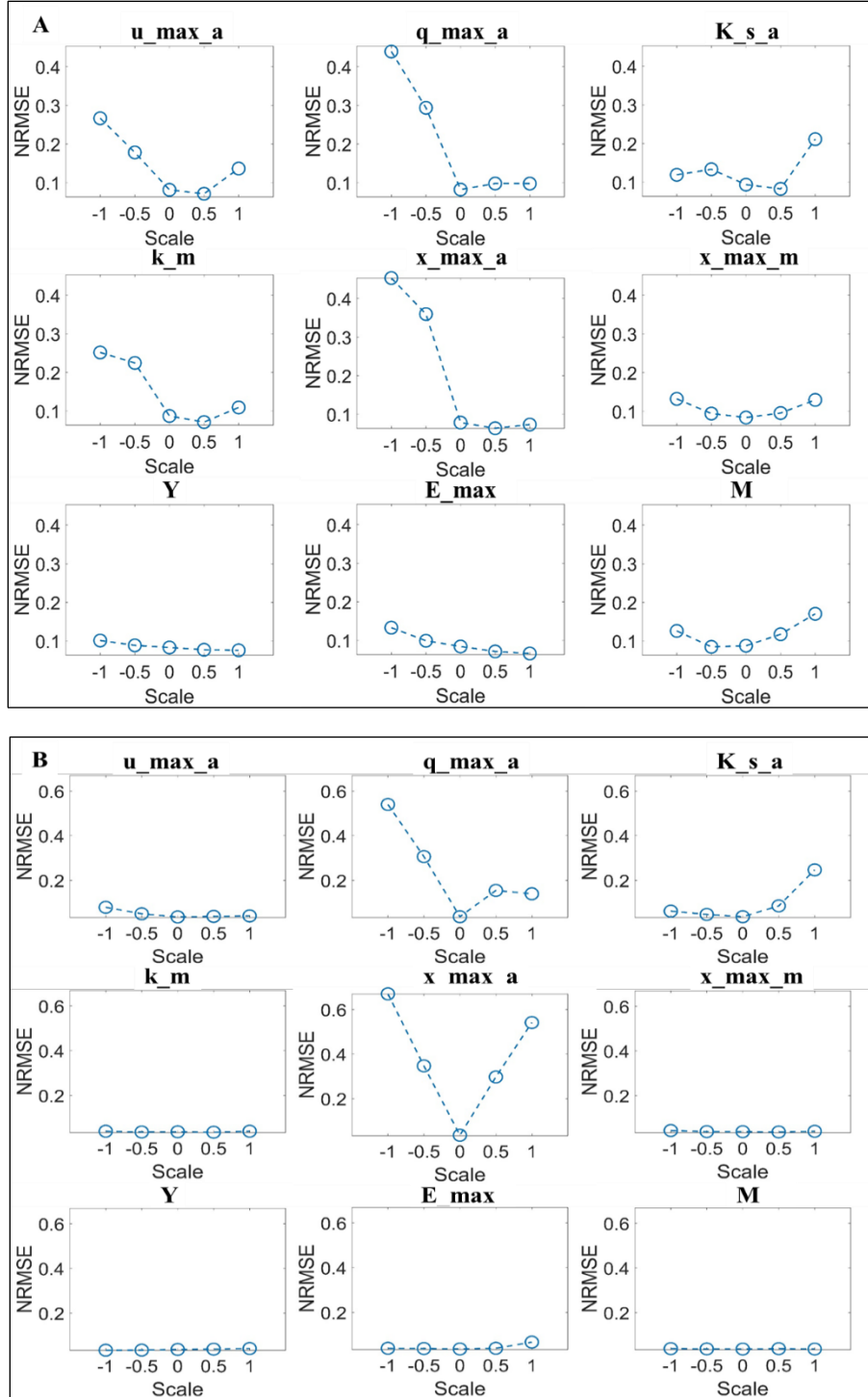


Figure 7.5 Normal_Region_90% sensitivity analysis for nine calibration parameters: (A) current generation sensitivity analysis; (B) organic removal efficiency sensitivity analysis. The larger changes in NRMSE under different scales are, the more important a calibration parameter is for the current output.

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7.3.4. Predication of optimized MFC design

The calibrated ensemble model was used to optimize the MFC design. The ranges of the input factors anolyte flow rate (x_1) (mL min^{-1}), influent acetate concentration (x_2) (g L^{-1}), and catholyte pump rate (x_3) (mL min^{-1}) in Eq. (7.5) were set as $1 < x_1 < 5$, $0.15 < x_2 < 0.80$, and $1 < x_3 < 100$, where the extrapolation range for x_1 , x_2 and x_3 was: $x_1 < 1.5$ or $x_1 > 4$; $x_2 < 0.26$ or $x_2 > 0.83$; $x_3 < 1.5$ or $x_3 > 75$, respectively. The input factor settings were optimized by varying the weight factor values $w = (0.1, 0.2, \dots, 0.9)$, where greater w indicates that the design has higher importance on current generation; vice versa. At each w , a set of points uniformly spread in the design space of the input factors were used as the initial searching points, and the “interior-point” algorithm was used for optimizing Eq. (5) [388]. Iterative local search was used to avoid local optimum for the highly nonlinear response surfaces (Figure 7.4) [387], and find the best candidate combination of the input factor settings (i.e., the optimal values of the x_1 , x_2 and x_3 values) given w .

Figure 7.6 summarizes the optimized input factor settings, the predicted current generation and organic removal efficiency values by varying the weight factor w . The optimized input factor settings are usually at the extrapolation region (such as $x_1 = 1.00$, $x_2 = 0.19$, $x_3 = 66.84$ at $w = 0.1$), which validates that the ensemble model for extrapolation and design is important for MFC scale up. Specifically, the increase of anode flow rate (x_1) and organic concentration (x_2) could enhance the current generation, accompanied with the low catholyte pumping flow rate (x_3) when $w > 0.5$. The prediction of abrupt current increase from 22.2 to 33.7 mA with w increased from 0.6 to 0.7 indicated that the simultaneous increase of anode flow rate from 1.00 to 1.74 mL min^{-1} and organic concentration from 0.22 to 0.40 g L^{-1} would greatly benefit the current generation. Relatively low catholyte pump rate was also predicted to favor the current generation (e.g., less than 31 mL min^{-1} when $w = 0.7 - 0.9$). Eventually, the current generation could increase to the peak value of 39.2 mA ($w = 0.9$), using the optimized design with an anode flow rate of 3.47 mL min^{-1} , an organic concentration of 0.71 g L^{-1} , and a catholyte pumping flow rate of 14.74 mL min^{-1} . The prediction results further proved that a higher organic loading rate could greatly increase the current generation (as shown in Figure 7.6). In addition, properly low catholyte pumping rate was strongly recommended not only for high current generation, but also for low energy pumping consumption [381]. The organic removal efficiency was predicted to be consistently 100% until w increased to

0.6 (Figure 7.6), suggesting that high organic removal efficiency was accompanied with low organic loading rates. A higher organic loading rate led to decreased organic removal efficiency, from 100% to 70% and further to 16%, when the organic loading rate increased from 0.32 to 1.00 and further to 3.55 kg m⁻³ day⁻¹, when $w = 0.6, 0.7, 0.9$, respectively. To achieve the best organic removal efficiency of 100% ($w = 0.1- 0.6$), the anode flow rate and organic concentration were better controlled at less than 1.04 mL min⁻¹, and 0.22 g L⁻¹, respectively, while the change of catholyte pumping flow rate had insignificant impact.

To validate that the optimal design can yield better MFC system performance than the measurement data, Figure 7.6 compares the input factor settings and the corresponding ensemble model prediction for the measurement data that yielded the smallest objective function value in Eq. (7.5) by varying the weight factor. Note that the optimization in Eq. (7.5) is a minimization problem, a smaller objective function value indicates a better input factor setting (the combination of anolyte flow rate (x_1), influent acetate concentration (x_2), and catholyte pump rate (x_3) values) towards high current generation and high organic removal efficiency. By comparing the objective function values yielded from the design and the measurement data (Eq. (7.5)), the design yields better (smaller) objective values than those of the measurement data at all weight factor values (Figure 7.6). For example, the objective function value in Eq. (7.5) at $w=0.1$ for the optimized design is -0.97, which is smaller than the objective function value (-0.53) for the measurement data. Therefore, the optimized design could indeed provide optimized input factor settings for MFC design optimization towards MFC performance improvement.

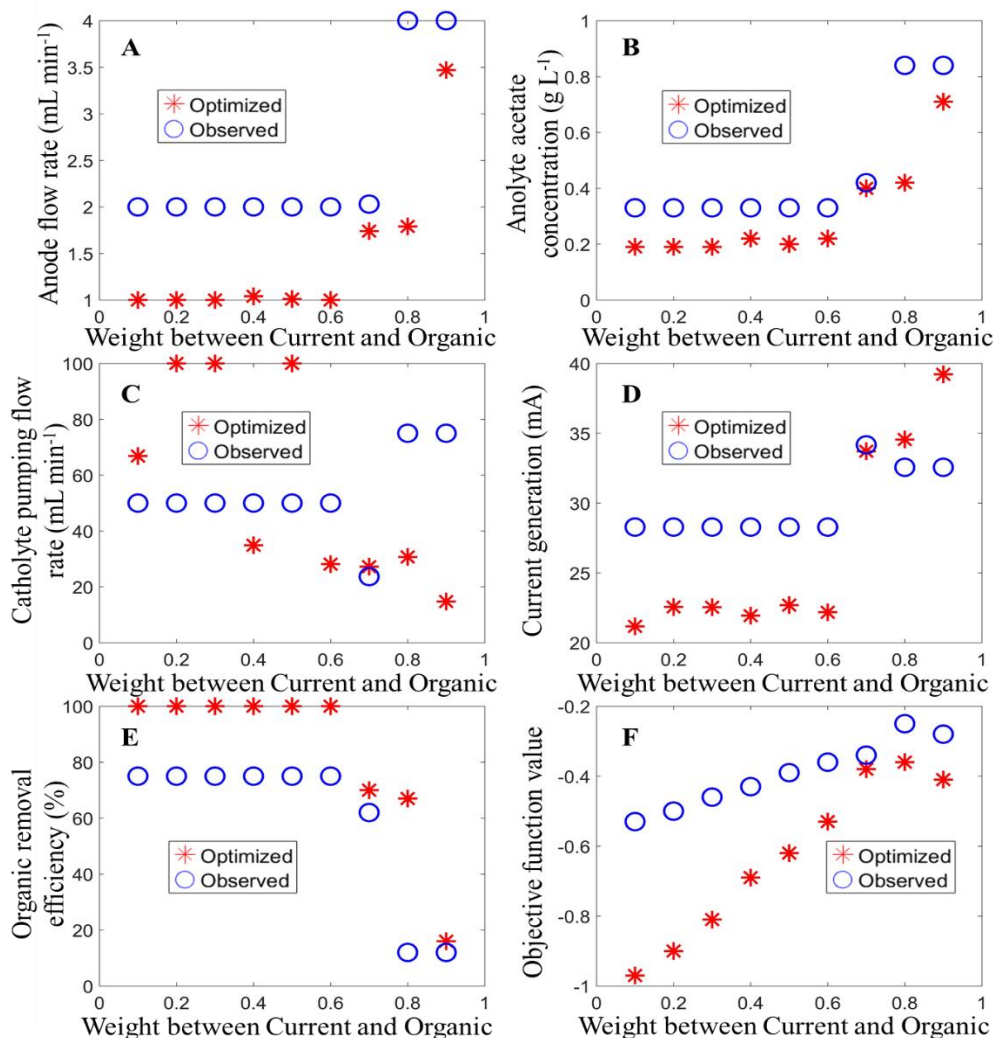


Figure 7.6 Input factor settings (A-C) and predicted output variables (D-E) from optimized MFCs design (Optimized) and measurement data with the best objective function value (Observed): (A) anode flow rate (mL min^{-1}), (B) anolyte acetate concentration (g L^{-1}), (C) catholyte pumping flow rate (mL min^{-1}), (D) current generation (mA), (E) organic removal efficiency (%), and (F) objective function value. The horizontal axis of each figure represents the weight between current generation and organic removal efficiency.

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7.3.5. Perspectives of ensemble modeling

Despite the superior performance of the ensemble model through integrating the merits of engineering and statistical principles for MFCs modeling and design, there are several challenges to address in future studies. First, the MFC model calibrated in this study considered five MFC modules as one system, and thus could not diagnose which MFC module was the limiting factor

for the entire system performance. This could be very important, considering that MFC scaling up will likely go with the strategy of multiple module design. To address this problem, the ensemble model may be extended to reveal the spatial relationship of MFC modules in the system and the variation in the calibration parameters in MFC modules. Other than building an ensemble model for the entire MFC system, a system of ensemble models will be built with self-diagnosis capability to achieve the optimized output for each MFC module. The computation issue for parameter estimation in the ensemble models system could be addressed by stochastic gradient MCMC [389]. Second, it is critical to ensure proper function of scaled MFCs, but it was reported that the MFC performance degraded in the long run [114]. Such a degradation effect has not been modeled mathematically. In the aspect of mathematical modeling for proper predication of MFC performance in long term operation, the biofouling effect, catalyst poisoning or inorganic fouling on cathode material with real wastewater treatment will need to be considered [32, 390, 391]. Thus, a specific component for system performance degradation will be included in the engineering model (e.g., parameter or equation to quantify the relation between cathodic biofouling and internal resistance), and the nonlinear general path model will be introduced to consider the degradation of the MFC in long-term operation [392]. As a result, the dynamic ensemble model considering the degradation effect could be built for life cycle assessment of long-term MFC operation. Third, in the present study the ensemble model was based on tubular MFCs, and its application to other MFC configurations or other bioelectrochemical systems (BES) such as microbial electrolysis cells (MECs), microbial desalination cells (MDC) and osmotic microbial fuel cells (OsMFC) warrants further efforts to integrate GP process components into the original engineering models for developing new ensemble model [165, 166, 374, 393]. New parameters will need to be calibrated, such as diffusion coefficient of salt in MDC model and permeability coefficient in OsMFC model. More factors will be counted into optimized input factor design such as salt concentration in desalination chamber in MDCs, or the impact of various types of wastewater on the MFC performance [88, 161, 166]. Finally, the constructed ensemble model can be used for online monitoring and control of MFC systems [372, 394]. For example, the spatial relationship of anolyte acetate concentration in serially connected MFCs in an MFC system can be monitored to identify the system problem in real time [372]. The input factor settings can also be adjusted in real time to meet the dynamic requirement of current generation and organic removal efficiency [395].

7.4 Conclusions

This paper proposes an ensemble model to integrate engineering model and data analytics for MFC system scale up. The ensemble model has exhibited the advantages of requiring fewer measurement data to achieve comparable modeling accuracy compared with the traditional statistical model under both the normal and extreme operation regions. The parameter calibration in the engineering model can be achieved by the ensemble model, where the labor-intensive model construction procedure is reduced. Given the weight between current generation and organic removal efficiency, the ensemble model can provide recommended input factor settings to achieve best current generation and organic removal efficiency for the tubular MFCs employed in this study. The ensemble model can provide useful guideline for scaling-up MFC system, and be applied to model other bioelectrochemical systems.

Chapter 8 Mathematical Modeling of the Dynamic Behavior of an Integrated Photo-bioelectrochemical System for Simultaneous Wastewater Treatment and Bioenergy Recovery

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Nomenclature

AEM	Anion exchange membrane
C_{AB-A}	Concentration of attached biomass in cathode (mg L^{-1})
C_{AB-S}	Concentration of suspended biomass in cathode (mg L^{-1})
C_{NH_4-N-a}	Effluent NH_4^+ -N concentration in the anode (mg L^{-1})
C_{NH_4-N-c}	Effluent NH_4^+ -N concentration in the cathode (mg L^{-1})
C_{NH_4-N-in}	Influent NH_4^+ -N concentration (mg L^{-1})
C_{NO_3-N-a}	Effluent NO_3^- -N concentration in the anode (mg L^{-1})
C_{NO_3-N-c}	Effluent NO_3^- -N concentration in the cathode (mg L^{-1})
C_{NO_3-N-in}	Influent NO_3^- -N concentration (mg L^{-1})
C_{P-c}	Effluent TP in the cathode (mg L^{-1})
DO	Dissolved oxygen concentration in the cathode (mg L^{-1})

D_r	Constant for organic reaction consumption by diffused oxygen in anode (dimensionless)
D_d	Fraction constant of diffused oxygen from cathode to anode (dimensionless)
D_p	Photosynthesis constant (day^{-1})
D_{cr-d}	Reaction constant of bacterial respiration in cathode at daytime (day^{-1})
D_{cr-n}	Reaction constant of bacterial respiration in cathode at night (day^{-1})
d_c	Conversion constant from decayed algal biomass to COD in cathode (dimensionless)
$E_{cathode}^o$	Standard cathode potential (V)
$E_{cathode}$	Actual cathode potential (V)
F	Flow rate (L day^{-1})
F_a	Faraday constant (C mol^{-1}) or (A s mol^{-1})
I	Current (A)
I_{NH_4-N-a}	Electrochemical reaction constant by using $\text{NH}_4^+\text{-N}$ as electron donor in anode (g mol^{-1})
K_{NH_4-N-a}	Constant for nitrification in anode for converting $\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$ (day^{-1})
K_{NO_3-N-a}	Constant for denitrification reaction in anode (L mg^{-1})
K_{C-AB}	Half saturation constants for carbon for biomass growth (mg L^{-1})

K_{NH_4-N-AB}	Half saturation constants for NH_4^+ -N for biomass growth ($mg\ L^{-1}$)
K_{NO_3-N-AB}	Half saturation constants for NO_3^- -N for biomass growth ($mg\ L^{-1}$)
K_{NO_3-N-cn}	Constant for denitrification reaction in cathode at night ($L\ mg^{-1}$)
K_{resp}	Conversion coefficient to remove COD by bacterial respiration in cathode (dimensionless)
K_{P-AB}	Half saturation constants for TP for biomass growth ($mg\ L^{-1}$)
K_H	Henry's constant for oxygen ($atm \cdot L\ mol^{-1}$)
k_{d-AB}	Decay constant of algal biomass in cathode (day^{-1})
M	Molar mass of oxygen ($g\ mol^{-1}$)
OP_{oxygen}	Oxygen overpotential (V)
OP_{conc}	Concentration overpotential (V)
$ODEs$	Ordinary differential equations
pO_2	Partial pressure of oxygen concentration (bar)
q_a	COD consumption rate by anodophilic bacteria (day^{-1})
q_m	COD consumption rate by methanogenic bacteria (day^{-1})

q_{S-d}	Carbon uptake constant by biomass in cathode at daytime (day^{-1})
q_{S-n}	Carbon uptake constant by biomass in cathode at night (day^{-1})
R	Ideal gas constant ($\text{J K}^{-1} \text{mol}^{-1}$)
R_{int}	Internal resistance (Ω)
R_{ext}	External resistance (Ω)
$RMSE$	Root mean square error (%)
S_a	COD concentration in anode (mg L^{-1})
S_c	COD concentration in cathode (mg L^{-1})
S_{in}	COD concentration in the influent (mg L^{-1})
T	Temperature (K)
TP	Total phosphorus (mg L^{-1})
V_a	Anode volume (L)
V_c	Cathode volume (L)
V_{oc}	Open circuit voltage (V)
x_a	Concentration of anodophilic bacteria in anode (mg L^{-1})

x_m	Concentration of methanogenic bacteria in anode (mg L^{-1})
θ	Electromigration coefficient of acetate (CH_3COO^-) from cathode to the anode ($\text{g mol}^{-1} \text{ dm}^{-2}$)
α	Electromigration coefficient of NO_3^- from cathode to the anode ($\text{g mol}^{-1} \text{ dm}^{-2}$)
$\mu_{\max-AB}$	Maximum specific growth rate of algal biomass in cathode (day^{-1})
φ_{COD}	Weighing factor for COD in cathode (dimensionless)
φ_{NH_4}	Weighing factor for NH_4^+ -N in cathode (dimensionless)
φ_{NO_3}	Weighing factor for NO_3^- -N in cathode (dimensionless)
φ_P	Weighing factor for TP in cathode (dimensionless)

8.1 Introduction

Wastewater treatment is a key process to address water pollution and protect water resources, but this process requires a large amount of energy [24]. To make wastewater treatment more sustainable, recovery of valuable resources such as energy and other value-added products has drawn broad interests and created necessity for developing new technologies [396]. Microbial fuel cells (MFCs) are an emerging treatment concept that can recover electric energy from organic contaminants in wastewater via microbial interaction with solid electron acceptors/donors [26, 28]. Despite the promise for organic removal and electricity generation, MFCs cannot effectively remove nutrients such as nitrogen and phosphorous [70], which are the key inorganic contaminants responsible for eutrophication. Algal bioreactors have been applied to treat wastewater with effective nutrient removal and simultaneous production of algal biomass that can be further processed to make bioenergy or other valuable products [397, 398]. Therefore, an integration of

algal bioreactors into MFCs holds promise to achieve the removal of both organic and nutrient compounds along with the production of bioelectricity and biomass [71], and such cooperation creates a new treatment technology called “integrated photo-bioelectrochemical (IPB)” system [3, 91].

In an IPB system, the MFC anode receives wastewater and degrades organic compounds with the production of electrons; the anode effluent containing nutrients (e.g. N and P) is sent to the MFC cathode, which also acts as an algal bioreactor. Multiple synergies can be realized through this interaction. For example, the dissolved oxygen produced by algae can be used for cathodic reactions to complete the electric circuit [399]; nutrients are removed from water and used for algal growth; CO₂ produced from anodic oxidation of organics can be used by algae; and both bioelectricity and algal biomass (to produce biofuel) can be generated [98, 99]. The feasibility of the IPB system has been demonstrated experimentally. It was found that 92.4% soluble COD (chemical oxygen demand), 63% total nitrogen (TN) and 82.3% total phosphorus (TP) could be removed, and the energy production (including electricity and biomass biofuel) could theoretically compensate for energy consumption of the IPB system [3]. The algal-bacterial community in the cathode can help nutrient removal in IPB, and certain photoautotrophs (e.g., *Leptolyngbya*) or chlorophytes (e.g., *Acutodesmus*) were found as significant species in the community [4].

Further understanding of the IPB system performance is important for its development and application in practice. The key questions to address include what and how input variables impact the system outputs (e.g., biomass growth, current generation), and how to achieve system optimization. However, only depending on experimental data and analysis is often insufficient and inconvenient to numerically and intuitively quantify the impact of various factors on the system performance [400], especially when these factors simultaneously take place and are strongly interdependent [401]. Mathematical modeling can be used as a powerful tool to quantitatively explain the relations between input and output parameters, and to ultimately provide optimization recommendation for system performance improvement (e.g., improve bioproduction of compounds) [402, 403]. The quantitative model can serve at least for two purposes: one is to develop a numerical standard to predict the system performance under investigation, and the other is to explain mechanisms and phenomena behind experimental observation [400]. Although many

mathematical models have been developed for MFCs to interpret the impact of various input factors (e.g., influent organic concentration) on the system output (e.g., current generation) [115, 161, 246], there has not been any model development for IPB systems.

This study for the first time developed a dynamic engineering model for the IPB system. Sensitivity analysis was applied to assist parameter determination, and then experimental data collected under various operating conditions were used to validate this model by using root-mean-square error (RMSE) as a fitness standard. The model development went through three stages: model fitting, model validation, and model prediction. The IPB model developed in this study is expected to provide new insight into the working mechanisms of an IPB system, and help to predict and optimize the dynamic performance of IPB outputs (i.e., biomass growth, current generation, dissolved oxygen and nutrient removal) in response to varied input factors (i.e., influent COD, NH_4^+ -N and TP concentration, flow rate and external resistance).

8.2 Materials and methods

8.2.1 IPB system setup

The IPB system consisted of a tubular MFC installed in a transparent tubular Plexiglass acrylic container that acted as both a cathode chamber and an algal bioreactor (Figure 8.1), according to a previous study [3]. The tubular MFC was made of anion exchange membrane (AEM, Ultrex CMI7000, Membranes International, Inc., GlenRock, NJ, USA) with a diameter of 4.9 cm and a height of 26 cm, resulting in an anodic working volume of 500 mL. A carbon brush was inserted inside as an anode and pretreated before use [404]. The cathode was made of carbon cloth coated with Pt/C powder ($\sim 0.3 \text{ mg Pt cm}^{-2}$). The carbon cloth wrapped the AEM with titanium wires in connection with the anode across an external resistor according to the experimental design shown in Table S1. The outer Plexiglass container had a diameter of 8 cm and a height of 29 cm, creating a cathodic liquid volume at 450 mL. Two fluorescent light tubes (40 W, 125 V, 4100 K, GE, CT, USA) were placed vertically beside the IPB system to provide illumination on a cycle of 12 h on and 12 h off schedule to simulate daytime and nighttime.

8.2.3 Measurement and analysis

The voltage of the IPB system was recorded every 5 min by using a digital multimeter (2700, Keithley Instruments, Inc., Cleveland, OH). The concentrations of soluble COD, NH_4^+ -N, NO_3^- -N, and TP were measured by using a DR/890 data logging colorimeter (Hach Company, Loveland, CO, USA). The pH was measured using a benchtop pH meter (Oakton Instruments, Vernon Hills, IL, USA). The dissolved oxygen (DO) was measured by using a DO meter (Hanna Instrument, Woonsocket, RI, USA). The cathodic algal biomass was determined from two sources, namely suspended biomass in the catholyte and attached biomass on the cathode (carbon cloth). The concentration of the suspended algal biomass in the cathode was measured by using a spectrophotometer (Spectronic Genesys 5; Spectronic Instruments, Rochester, NY) for absorbance at 680 nm, to indicate the biomass growth condition. Gentle mixing, without disturbing the attached biomass on cathode, was provided to the catholyte before catholyte collection and measurement for algal biomass. The concentration of the suspended biomass was measured based on a standard curve shown in Figure G1. The mass of the attached biomass was measured by scraping the biomass from the carbon cloth after finishing all experiments, and then converted to the suspended biomass concentration per unit cathode volume for model calculation.

8.2.4 Model development

8.2.4.1 MFC anode model

Medium recirculation was applied to provide close to homogenous mixing in the anode. Hence, ordinary differential equations (ODEs) could be developed to quantify the mass balance in the MFC anode based on multiplicative Monod kinetics for the microbial growth (anodophilic and methanogenic bacteria), intracellular redox mediator turnover, and organic degradation as explained in previous studies [150]. Multiple physiochemical and biological processes are considered in the model formulation (e.g., oxygen diffusion and ionic electromigration through AEM, anaerobic denitrification). The ODE expression formats are generally listed as (use Eq.8.1 as example): rate of mass change anode (e.g., COD change, $\frac{dS_a}{dt}$) = (dilution effect of influent and effluent rate versus anode volume on mass change (e.g., COD dilution and oxidation by diffused oxygen from cathode to anode, $\frac{F}{V_a}(S_{in} - S_a - D_r \cdot D_d \cdot DO)$)) - (mass consumption by bacterial degradation (e.g., substrate degradation by electrogenic and methanogenic bacteria, $-q_a \cdot x_a -$

$q_m \cdot x_m$)) $\pm$ (mass consumption or gain by biochemical reaction (e.g., denitrification for COD consumption, $0.587 \cdot K_{NO_3-N-a} \cdot C_{NO_3-N-a} \cdot S_a$)) + (anion mass transfer from cathode to anode driven by electron migration, (e.g., CH_3COO^- anion, $\frac{\theta \cdot I \cdot A}{F_a \cdot V_a}$)). More details are explained below, based on the assumptions shown in Table G2. The ODEs for mass balance of anodic microorganisms (anodophilic and methanogenic) and intracellular redox mediator are formulated based on the understanding as explained in previous work [166], and are listed in Eqs. G1 to G3. All mathematical symbols used in this study are defined in the nomenclature.

The ODE for mass balance of COD (S_a), NH_4^+-N (C_{NH_4-N-a}), and $NO_3^- -N$ (C_{NO_3-N-a}) in the anode can be expressed in Eqs. 8.1 to 8.3, respectively,

$$\frac{dS_a}{dt} = \frac{F}{V_a} (S_{in} - S_a - D_r \cdot D_d \cdot DO) - q_a \cdot x_a - q_m \cdot x_m - 0.587 \cdot K_{NO_3-N-a} \cdot C_{NO_3-N-a} \cdot S_a + \frac{\theta \cdot I \cdot A}{F_a \cdot V_a} \quad (\text{Eq. 8.1})$$

$$\frac{dC_{NH_4-N-a}}{dt} = \frac{F}{V_a} (C_{NH_4-N-in} - C_{NH_4-N-a}) - K_{NH_4-N-a} \cdot D_d \cdot DO - \frac{I_{NH_4-N-a} \cdot I}{8 \cdot F_a \cdot V_a} \quad (\text{Eq. 8.2})$$

$$\frac{dC_{NO_3-N-a}}{dt} = \frac{F}{V_a} (C_{NO_3-N-in} - C_{NO_3-N-a}) + K_{NH_4-N-a} \cdot D_d \cdot DO - K_{NO_3-N-a} \cdot C_{NO_3-N-a} \cdot S_a + \frac{\alpha \cdot I \cdot A}{F_a \cdot V_a} + \frac{I_{NH_4-N-a} \cdot I}{8 \cdot F_a \cdot V_a} \quad (\text{Eq. 8.3})$$

The term $D_d \cdot DO$ in Eqs. 8.1 to 8.3 quantifies the amount of diffused oxygen from the cathode to the anode. Its product with D_r in Eq. 1 expresses the fraction of total anodic COD to react with diffused oxygen. $K_{NH_4-N-a} \cdot D_d \cdot DO$ in Eqs. 8.2 and 8.3 represents mass change on N by nitrification for the consumption of NH_4^+-N and gain of $NO_3^- -N$, respectively. The transportation of various ionic species across AEM also needs to be considered for charge balance in the anode and the cathode. In this study, NO_3^- , CH_3COO^- (COD), and PO_4^{3-} (TP) are anions available for transportation, and the mathematical term ($\frac{I \cdot A}{F_a \cdot V_a}$) is employed to represent the total amount of ions migrated [407]. α and θ are the constants for the fraction of NO_3^- and CH_3COO^- in total transported ions across AEM. Denitrification reaction is also considered, and the term ($K_{NO_3-N-a} \cdot C_{NO_3-N-a} \cdot S_a$) is used to quantify the integrated effects of both $NO_3^- -N$ and COD concentration on the rate of $NO_3^- -N$ reduction (note that “0.587” is the mass stoichiometric ratio of COD versus $NO_3^- -N$ [408]). The term ($\frac{I_{NH_4-N-a} \cdot I}{8 \cdot F_a \cdot V_a}$) is used to express the rate of electricity generation from ammonium (NH_4^+-N) oxidation. ODE for dynamic behavior of TP in the anode

(considering release or uptake by polyphosphate accumulating organisms (PAOs), and electromigration) is presented in similar formula in Eq. G4.

8.2.4.2 MFC cathode model

Ideal mixing is assumed for the cathode, and ODEs are developed considering dynamic behavior of suspended algal biomass, dissolved oxygen, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and TP in the cathode, based on assumptions listed in Table G2. The growth of algal biomass is affected by elements C, N and P. A weighted model is formulated to determine the limiting factors (e.g. COD, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, TP) on the growth rate of algal biomass ($\frac{dC_{AB-S}}{dt}$) in the cathode referring to the previous works [409, 410]:

$$\frac{dC_{AB-S}}{dt} = \mu_{max-AB} \cdot (C_{AB-S} + C_{AB-A}) \cdot \min \left[\varphi_{COD} \cdot \frac{S_c}{S_c + K_{C-AB}}, \varphi_{NH_4} \cdot \frac{C_{NH_4-N-c}}{C_{NH_4-N-c} + K_{NH_4-N-AB}}, \varphi_{NO_3} \cdot \frac{C_{NO_3-N-c}}{C_{NO_3-N-c} + K_{NO_3-N-AB}}, \varphi_P \cdot \frac{C_{P-c}}{C_{P-c} + K_{P-AB}} \right] - \frac{F}{V_C} \cdot C_{AB-A} - k_{d-AB} \cdot (C_{AB-S} + C_{AB-A}) \quad (\text{Eq. 8.4})$$

It is assumed in Eq. 8.4 that the rate of algae growth is limited by the minimum value of four factors related to respective concentration of COD, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and TP in the cathode. φ is the weighing factor to adjust the quantitative relationship among four factors, whose values are obtained by model fitting. The reasons to link COD to biomass growth ($\varphi_{COD} \cdot \frac{S_c}{S_c + K_{C-AB}}$ in Eq. 8.4) include: 1) some algae can consume organic carbon to grow [78], and 2) higher COD encourages a higher bacterial activity in the cathode and thus more CO_2 is produced for algal uptake and growth [411]. In Eq. 4, $\mu_{max-AB} \cdot (C_{AB-S} + C_{AB-A})$ represents the basic determinant for biomass growth rate. Suspended algae (C_{AB-S}) and biomass attached on the carbon cloth (C_{AB-A}) are taken into account, and the ODE is formulated to reflect the change rate of the suspended algal biomass ($\frac{dC_{AB-S}}{dt}$), assuming that the concentration of the attached biomass is saturated and can be set as constant (measured as 103.4 mg L^{-1}), from which the new algal growth should detach and become suspended biomass.

Because the dynamic behavior of biomass (algae and bacteria) to produce or consume DO, remove COD by bacterial respiration, and remove nutrients by algal uptake is different between daytime and nighttime, the ODEs are constructed differently with certain parameters varying referring to

the previous study [412]. The ODEs for mass balance of the cathodic DO changing rate ($\frac{dDO}{dt}$) are listed and explained in Eqs. 8.5.1 and 8.5.2:

$$\frac{dDO}{dt} = D_p \cdot (C_{AB-S} + C_{AB-A}) - 2 \cdot K_{NH_4-N-c} \cdot DO - D_{cr-d} \cdot DO - \frac{F}{V_c} \cdot (DO + D_d \cdot DO) \quad (\text{Eq. 8.5.1})$$

(for daytime)

$$\frac{dDO}{dt} = -2 \cdot K_{NH_4-N-c} \cdot DO - D_{cr-n} \cdot DO - \frac{F}{V_c} \cdot (DO + D_d \cdot DO) \quad (\text{Eq. 8.5.2}) \quad (\text{for night})$$

The term ($D_p \cdot (C_{AB-S} + C_{AB-A})$) represents the photosynthetic oxygen production rate during daytime; the term ($2 \cdot K_{NH_4-N-c} \cdot DO$) is the oxygen consumption due to nitrification; ($D_{cr-d} \cdot DO$, $D_{cr-n} \cdot DO$) represents the oxygen loss due to bacterial respiration during daytime or night respectively; $-\left(\frac{F}{V_c} \cdot (DO + D_d \cdot DO)\right)$ represents the oxygen loss due to dilution effect of effluent flowing out of the cathode and concentration-gradient diffusion from cathode to anode.

The mass balance of COD (S_c) in the cathode is listed in Eqs. 8.6.1 and 8.6.2:

$$\frac{dS_c}{dt} = \frac{F}{V_c} \cdot (S_a - S_c) - q_{S-d} \cdot (C_{AB-S} + C_{AB-A}) - K_{resp} \cdot D_{cr-d} \cdot DO - \frac{\theta \cdot I \cdot A}{F_a \cdot V_c} + d_c \cdot k_{d-AB} \cdot (C_{AB-S} + C_{AB-A}) \quad (\text{Eq. 8.6.1}) \quad (\text{for daytime})$$

$$\frac{dS_c}{dt} = \frac{F}{V_c} \cdot (S_a - S_c) - q_{S-n} \cdot (C_{AB-S} + C_{AB-A}) - 0.587 \cdot K_{NO_3-N-cn} \cdot C_{NO_3-N-c} \cdot S_c - K_{resp} \cdot D_{cr-n} \cdot DO - \frac{\theta \cdot I \cdot A}{F_a \cdot V_c} + d_c \cdot k_{d-AB} \cdot (C_{AB-S} + C_{AB-A}) \quad (\text{Eq. 8.6.2}) \quad (\text{for night})$$

The term ($\frac{F}{V_c} \cdot (S_a - S_c)$) represents the dilution effect of COD attributed to anode and cathode effluent in cathode; the terms ($q_{S-d}/q_{S-n} \cdot (C_{AB-S} + C_{AB-A})$) and ($K_{resp} \cdot D_{cr-d}/D_{cr-n} \cdot DO$) represent the carbon uptake by heterotrophic or mixotrophic algae and degradation by bacterial respiration during daytime and night respectively; the term ($d_c \cdot k_{d-AB} \cdot (C_{AB-S} + C_{AB-A})$) represents conversion of decay biomass to COD in cathode; $\frac{\theta \cdot I \cdot A}{F_a \cdot V_c}$ represents anion migration loss of CH_3COO^- migrating to anode; $0.587 \cdot K_{NO_3-N-cn} \cdot C_{NO_3-N-c} \cdot S_c$ is the carbon consumption due to denitrification affected by both carbon and NO_3^- concentration.

Likewise, the ODEs for dynamic behavior of NH_4^+-N , NO_3^--N , and TP also follow similar equation structure considering nutrient uptake by algal biomass, bacterial nitrification and denitrification, and ionic electromigration through AEM based on the assumptions described in Table G2, which are expressed in Eqs. G5 to G7.

8.2.4.3 Current generation model

The fundamental model frame for overall current generation in an IPB system is described in Eq. G8. The difference from a previous model frame [166] is that the present model considers the overpotential limited by oxygen concentration in the cathode, because the cathodic oxygen strongly varies between daytime and nighttime and influences the cathodic potential. The cathode potential is affected by the cathodic pH and oxygen concentration as described by $E_{cathode} = E_{cathode}^o - \frac{R \cdot T}{4 \cdot F} \ln \left(\frac{1}{pO_2 \cdot [H^+]^4} \right)$ [28]. The partial pressure of oxygen concentration (pO_2) has more impact than pH on the cathode potential based on the preliminary results. Thus, the term $\frac{R \cdot T}{4 \cdot F} \ln \left(\frac{1}{pO_2 \cdot [H^+]^4} \right)$ is perceived as a sole source of the cathodic overpotential (OP_{oxygen}) affected only by oxygen concentration variation, while assuming pH as a constant. The descriptive equations are listed in Eqs. 8.7 and 8.8 according to the previous works [28, 150, 166], and the assisting equations to calculate open circuit voltage (V_{OC}) and concentration overpotential (OP_{conc}) are listed in the Appendix G:

$$OP_{oxygen} = \frac{R \cdot T}{4 \cdot F_a} \ln \frac{M}{K_H \cdot DO \cdot [H^+]^4} \quad (\text{Eq. 8.7})$$

$$I = \frac{V_{OC} - OP_{conc} - OP_{oxygen}}{R_{ext} + R_{int}} \quad (\text{Eq. 8.8})$$

Eq. 8.8 is final equation derived from Eq. G8 for current generation (I) affected by V_{OC} , OP_{conc} , OP_{oxygen} together. Henry's law is employed to correlate dissolved oxygen to pO_2 ($K_H = 769.23$, $M=32$). pH is set at 8.25 ($[H^+] = 10^{-8.25}$), an average between daytime and nighttime. R_{int} is the internal resistance calculated based on Appendix G, and R_{ext} is the external resistance (Ω) as designed in Table G1.

8.2.4.4 Parameter determination

The initial values of most model parameters used in the course of parameter determination were adapted from our previous work [166]. To simplify the parameter determination process, the most influential parameters were determined in the first place. Sensitivity analysis was used as an effective tool to quantify the weight of the effect of each parameter on the model output according to the methods described in the previous studies (more details are described in Appendix G) [161, 166]. Briefly, one parameter was changed at a time in the course of sensitivity analysis, and higher output variation indicated more influence of a parameter. The parameter determination process

was further assisted by comparing the experimental and predictive values in terms of RMSE standard (more details are described in Appendix G) [161, 166]. Lower RMSE indicates a better fit of the model to the experimental data, and RMSE below 20% is often acceptable for the model fitting and validation [161].

8.3. Result and Discussion

8.3.1 Sensitivity analysis

All the parameters were determined with the assistance of sensitivity analysis for RMSE minimization. The parameters with top 10 sensitivity are listed in a descending order (Table G3), in which all parameters were normalized by the largest sensitivity value, i.e., the maximum specific growth rate of anodophilic microorganisms (μ_{max-a}). The sensitivity analysis not only helps adjust the parameter, but also gives quantitative indication for parameter impact on the system performance. For example, μ_{max-a} is strongly related to the activity of anodophilic microorganisms for current generation and ion migration through AEM. Another parameter, μ_{max-AB} , would also have strong impact on biomass growth and nutrient uptake into the biomass. High sensitivity of K_{NH_4-N-a} and D_d indirectly proves that oxygen diffusion into the anode should be considered for model analysis, because the diffused oxygen can compete with the anode for electron donors to reduce current generation [28]. Since these parameters could have strong impact on the model performance, they were given priority during model fitting. On the contrary, the sensitivity of parameters with regard to ionic migration was lower than many other parameters (Table G3), indicating that the ion emigration through AEM (e.g., CH_3COO^- , NO_3^- , PO_4^{3-}) might have little impact on the model performance.

8.3.2 Model fitting

There are 53 model parameters in need of determination, and their values were calibrated by comparing the model output against experimental output with the same input variables. In the experiment, the influent COD was varied from 270 to 154, 77 and then back to 270 mg L⁻¹ in the anode influent (Table G1). The significant effect of the influent COD input was observed on the output experimental parameters such as algal biomass, dissolved oxygen, COD, NH_4^+-N , $NO_3^- -N$, and TP in the anode and cathode effluent (Figures 8.2 and 8.3). Therefore, the influent COD values

were fed into the model, and unknown parameters were adjusted to fit the model output with the experimental outputs described above. In the course of model fitting, the initial values of most unknown parameters were adapted from our previous works [166], and the final parameter values were determined by using RMSE as a standard. The results of all parameter determination are shown in Table G4.

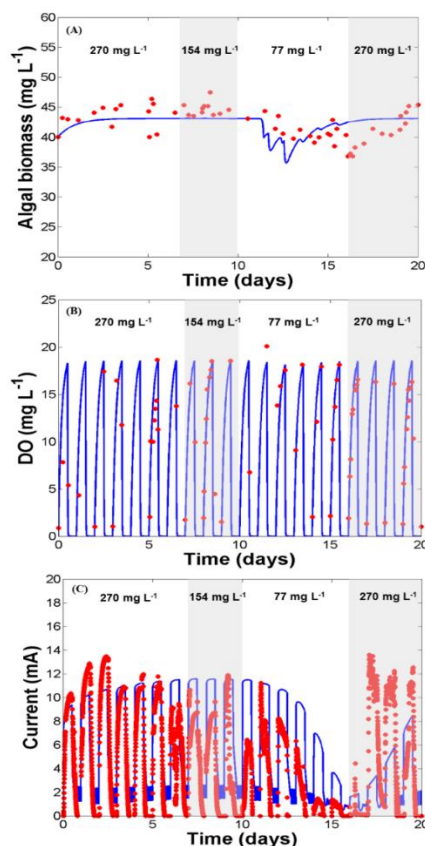


Figure 8.2 Model fitting for main output targets when the influent COD concentration was changed from 270 to 154, 77 and then back to 270 mg L⁻¹: (A) suspended algal biomass concentration in the cathode; (B) dissolved oxygen concentration in the cathode; and (C) current generation. Note: blue lines represent the simulation results and red dots represent the experimental data.

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As shown in Figures 8.2 and G2, the calibrated model output agreed well with the experimental output for main targets (e.g. suspended biomass concentration, DO and current generation). The model predicted that the algal biomass concentration was initially stabilized around 43 mg L⁻¹, and then gradually decreased to 38 mg L⁻¹ when the influent COD decreased to 77 mg L⁻¹ on day 10. Following that, the algal biomass recovered to 43 mg L⁻¹ when the COD returned to 277 mg L⁻¹,

which fit well with the experimental results having low RMSE of 5.6 % (Figure 8.2A and Table 8.1). The impact of the influent COD on the biomass growth indicated that the algae had the nutritional mode of heterotrophy or mixotrophy [413]. However, the delayed decrease or increase in algal biomass in the experiment was observed as compared to the model prediction, indicating the slower response of the actual experiment to different operational conditions than the model simulation (Figure 8.2A). This is likely related to the fact that the model has simplified a complex system by considering only a limited number of factors affecting biomass growth in Eq. 8.4, and the cathode was assumed to be homogenous, meaning that the change of operational input would immediately change the entire cathode condition. As a consequence, mathematical model for algal biomass showed higher response sensitivity to the input change than in actual conditions.

Table 8.1 RMSE of different outputs under different targets on the operational input

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	Model Output										
Model Input	I	AB	DO	A(C)	A(NH ₄ ⁺)	A(NO ₃ ⁻)	A(P)	C(C)	C(NH ₄ ⁺)	C(NO ₃ ⁻)	C(P)
COD	0.2%	5.6%	5.4%	2.3%	0.5%	26.8%	2.7%	11.8%	15.1%	14.7%	5.3%
External resistance	0.4%	8.2%	2.7%	2.0%	0.7%	41.3%	6.2%	7.7%	9.8%	16.3%	4.4%
Flow rate	0.5%	11.6%	9.7%	0.7%	0.5%	36.2%	4.1%	6.1%	10.7%	17.2%	6.9%
NH ₄ ⁺ -N	0.4%	6.3%	2.3%	1.5%	0.5%	42.2%	6.2%	7.9%	14.3%	15.3%	7.4%
TP	0.4%	5.8%	2.4%	1.2%	0.4%	42.8%	3.4%	7.8%	10.9%	19.4%	7.5%

Note: “I” represents current; “AB” represents suspended algal biomass concentration in cathode; “DO” represents dissolved oxygen; letters “A” and “C” before the bracket represents anode and cathode; letters “C”, “NH₄⁺”, “NO₃⁻” and “P” represent COD, NH₄⁺-N, NO₃⁻-N, and TP, respectively.

The simulated DO profile agreed well with the experimental results (Figure 8.2B), and achieved small RMSE of 5.4 % (Table 8.1). The calibrated model was capable of simulating the DO increase, which started at the beginning of illumination period, and DO reached the maximum value of 18 mg L⁻¹ at the end of illumination period, in synchronization with the moment at on/off of algal photosynthesis between daytime and night shift. The simulated current agreed in line with the experimental data (Figure 8.2C). The model predicted that the current reached the maximum of 11.5 mA with a sufficient supply of COD at 270 mg L⁻¹, and then gradually decreased to about 2.0

mA after decreasing the influent COD concentration to 77 mg L⁻¹, followed by current resumption to 11.5 mA when the COD concentration returned to 270 mg L⁻¹ (Figure 8.2C). In general, the model quantitatively captured the simulated current profile with a very low RMSE of 0.2 % (Table 8.1). The actual experiment had a lower minimal current of ~ 1 mA, likely due to the biofilm formation on the cathode, retarding the oxygen diffusion to the active sites and in turn impeding the current generation, which was not taken into account in the model formulation [414].

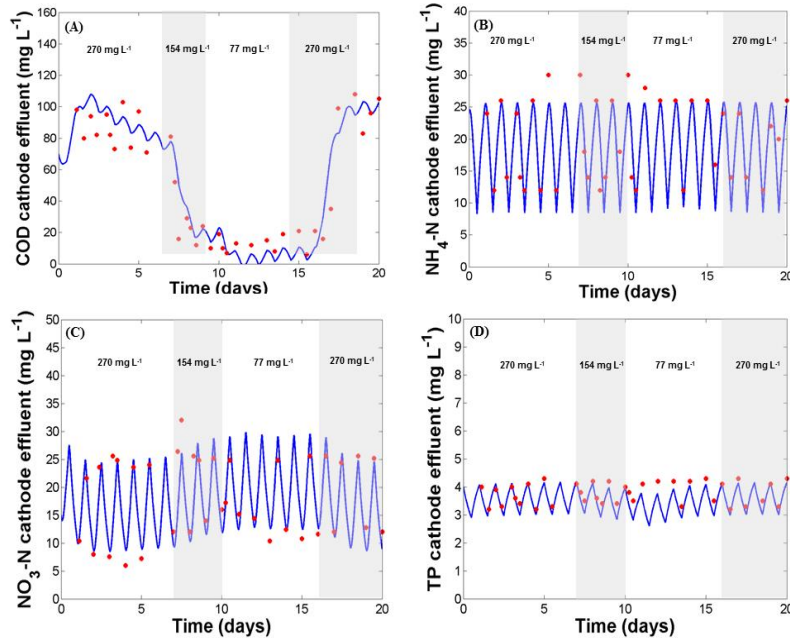


Figure 8.3 Model fitting for the nutrients when the influent COD was changed from 270 to 154, 77 and then back to 270 mg L⁻¹: (A) cathodic COD; (B) NH₄⁺-N; (C) NO₃⁻-N; and (D) TP. Note: blue lines represent the simulation results and red dots represent the experimental data.

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The output of the calibrated model was also in very good agreement with the effluent concentrations of COD, NH₄⁺-N, NO₃⁻-N and TP as shown in Figures 8.3 and G2 with RMSE <20% (Table 8.1). It was found that variation of the COD in the anode and the cathode followed an opposite trend to that of DO (Figures 8.2B, 8.3A and G2), indicating the impact of oxygen diffusion through AEM to promote organic degradation in the anode. The reversed variation trend of cathodic NH₄⁺-N and NO₃⁻-N suggested that nitrification was the main mechanism for ammonium conversion (Figures 8.3B and C). TP reduction was mainly accomplished via algal

uptake, while migration of phosphate ions across AEM had a limited effect (Figures 8.3D and G2), as suggested by sensitivity analysis showing that ionic migration was relatively insignificant.

8.3.3 Model validation

In order to confirm the validity of those calibrated parameters in Table G4 for the model simulation and to verify the model predictability, four other individual experiment inputs, including external resistance, flow rate, influent NH_4^+ -N and TP concentration, were also fed into the calibrated model (Table G1). Only those previously calibrated parameters were used for model validation (Table G4).

The first validation input was the external resistance that was changed from 8 to 100, 1 and then back to 8 Ω (Table G1). Both model prediction and experimental results showed that the variation of the external resistance had little effect on the biomass concentration that was stabilized around 43 mg L^{-1} (Figure 8.4A), but the current was obviously influenced (Figure 8.4B). The model prediction well fitted the experimental data with low RMSE (Table 8.1). The validation results of other outputs affected by the external resistance were shown in Figure G3.

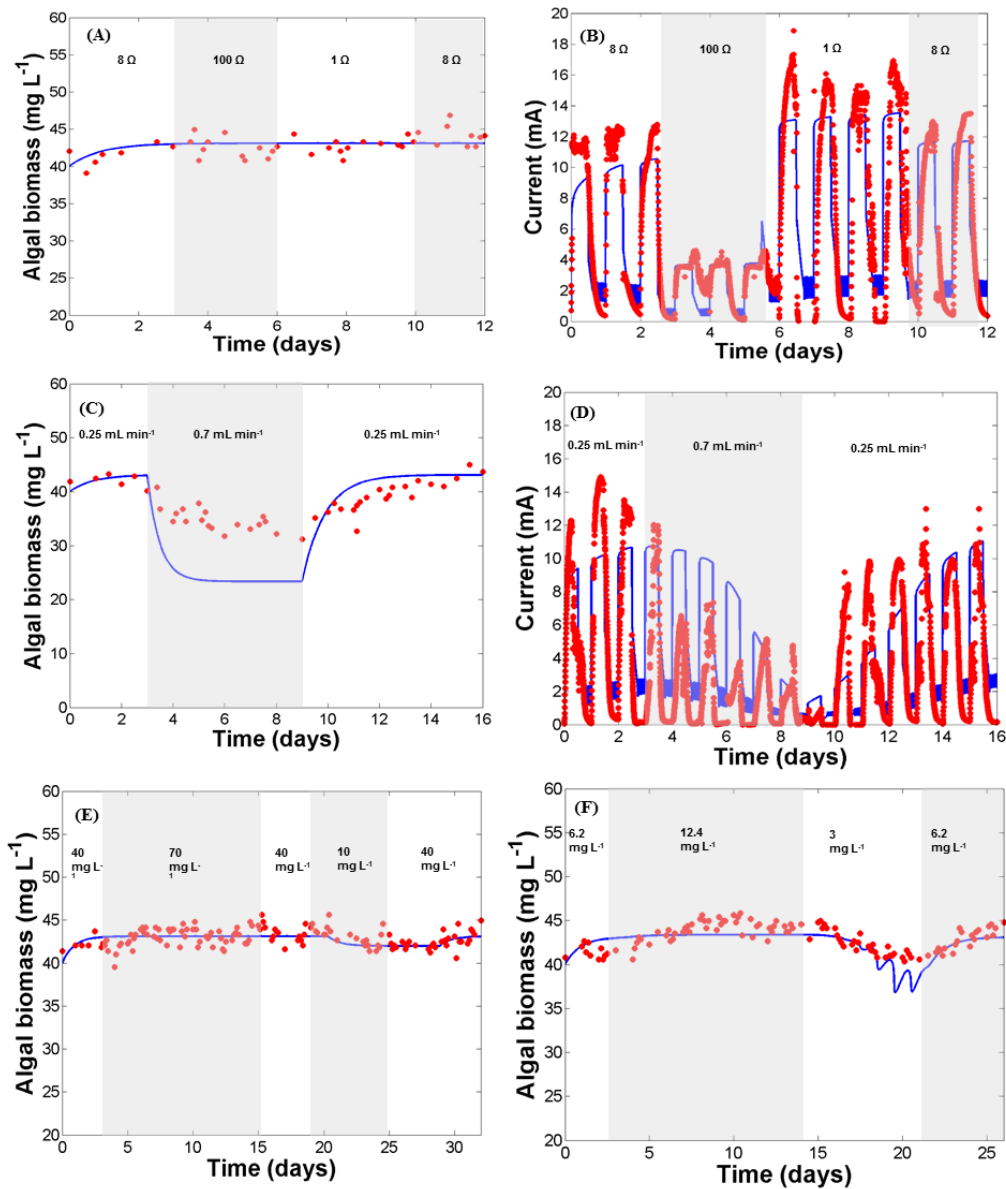


Figure 8.4 Model validation for algal biomass growth and current generation under varied operating conditions: (A) suspended biomass concentration and (B) current generation, when the external resistance was changed from 8 to 100, 1 and back to 8 Ω ; (C) suspended biomass concentration and (D) current generation, when the flow rate was changed from 0.25 to 0.7, and back to 0.25 mL min⁻¹; (E) suspended biomass concentration, when the influent NH_4^+ -N concentration was changed from 40 to 70, 40, 10 and then back to 40 mg L⁻¹; (F) suspended biomass concentration, when the influent TP concentration was changed from 6.2 to 12.4, 3 and then back to 6.2 mg L⁻¹. Note: blue lines represent the simulation results and red dots represent the experimental data.

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The second validation input was the flow rate, which varied from 0.25 to 0.7 and then back to 0.25 mL min⁻¹. This change clearly affected both the algal biomass concentration and current generation (Figures 8.4C and D), which were well predicted with satisfactory RMSE (Table 8.1 and Figure G4). The increase in the flow rate should increase the algal growth rate because of increased substrate and nutrient loading rate, but it also decreases the retention time of the suspended biomass by washing them out and thus allows less time for algae to grow. Consequently, the underestimation of algal biomass concentration at a higher flow rate (0.7 mL min⁻¹) was likely due to the stronger washout effect in the cathode, which was also observed in a previous work [415]; while the higher biomass concentration measured in the experiment was possibly the result of detached biomass from the cathode surface contributed by the faster growth of those attached biomass, which was not considered in the model formulation.

The third and fourth inputs used for model validation were the NH₄⁺-N and TP concentrations in the influent. NH₄⁺-N was changed from 40 to 70, 40, 10, and then back to 40 mg L⁻¹. TP was changed from 6.2 to 12.4, 3, and finally back to 6.2 mg L⁻¹. The varied NH₄⁺-N input had a negligible impact on the simulated biomass concentration (Figure 8.4E), and the NH₄⁺-N concentration (10 mg L⁻¹) was not low enough to limit biomass growth rate. Although the model output showed that low influent TP (3 mg L⁻¹) could decrease the biomass concentration to the minimum value of 36 mg L⁻¹, the experimental biomass concentration only decreased to 41 mg L⁻¹ (Figure 8.4F). Both model prediction and experiment results showed that varying NH₄⁺-N or TP had little impact on current generation (Figures G5 and G6), because they were not electron donors. The model prediction of the anodic NO₃⁻-N had exceptionally large RMSE (> 20%, Table 8.1), possibly because the colorimeter in this study had low detection sensitivity, only showing concentration of NO₃⁻-N with one decimal place, and the actual NO₃⁻-N concentration was at extremely low level (e.g., 0.1 to 0.5 mg L⁻¹). Thus, the relatively high RMSE of NO₃⁻ would be difficult to be avoided because the low experimental value in the equation denominator could greatly enlarge the relative error (Eq. G9 in Appendix G).

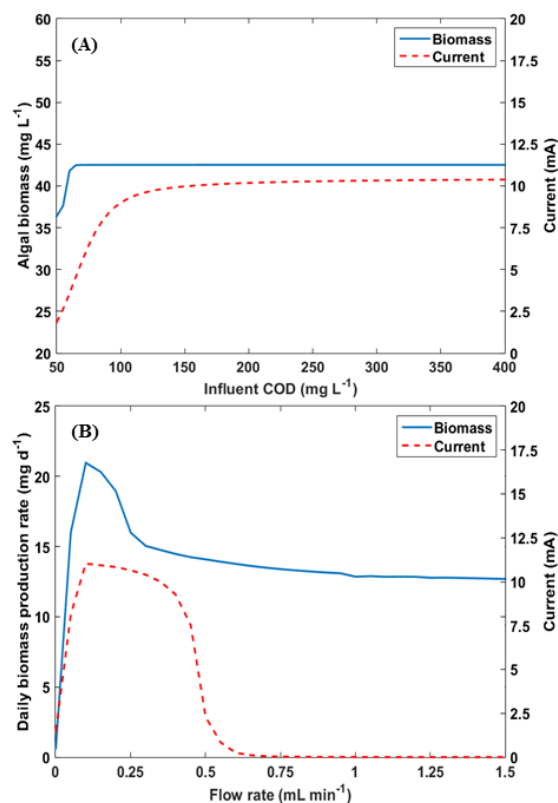


Figure 8.5 Model prediction with one factor variation to indicate the parameter impact on the suspended biomass concentration and current generation: (A) the influent COD; and (B) the flow rate.

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8.3.4 Model prediction

8.3.4.1 Effects of individual input parameter

The model validated above was employed to predict the biomass and current generation as a function of operational inputs (i.e., influent COD and flow rate) in a broad range beyond the experimental condition. One operational input was varied at a time to predict the steady-state output. The prediction range for COD input was chosen from 50 to 400 mg L^{-1} . The growth of the algal biomass reaches 43 mg L^{-1} after the COD concentration increases to 75 mg L^{-1} and there is no further increase despite a higher COD input; current generation increases with increasing COD concentration and reaches a plateau after 150 mg L^{-1} (Figure 8.5A), likely due to the oversupply of organic compounds that cannot be effectively converted to electricity by the anodophilic bacteria. In addition, the model considers the inhibitory factor of oxygen overpotential on current

generation, and thus the overpotential limits the further current increase at the end of the illumination period, even with a higher COD concentration.

The prediction range for the anolyte flow rate was chosen from 0 to 1.5 mL min⁻¹. An increase in the anolyte flow rate will shorten the retention time of the suspended biomass. At a steady state, the daily growth rate of the suspended biomass should be equal to the daily biomass production rate as calculated by flow rate × suspended biomass concentration. Hence, as the anolyte flow rate increases from 0 to 0.1 mL min⁻¹, the daily biomass production rate also increases from 0 to 21 mg d⁻¹ as shown in Figure 8.5B. However, once the flow rate exceeds a critical value, namely 0.1 mL min⁻¹ in this simulation, the maximum possible daily growth rate of suspended biomass (22 mg d⁻¹) will be exceeded. Consequently, the daily biomass growth will not be able to catch up with the daily biomass washed out of the IPB. As a result, the daily biomass production rate drops with further flow rate increase (Figure 8.5B). The reason why the daily biomass production rate does not drop to zero can be ascribed to the contribution of the detached biomass from the cathode to the suspended biomass. The simulated current profile follows that of the biomass (Figure 8.5B), i.e. it increases from 0 to a maximum value of 10 mA with the flow rate increase from 0 to 0.1 mL min⁻¹. After that, current decreases all the way to almost zero with further flow rate increase, likely due to the biofilm formation on the cathode (because of the increased organic loading rate that stimulates the growth of heterotrophic organisms) impeding the oxygen diffusion to the active sites for current generation. In addition, it has been well-known that strong washout creates a selection pressure in favor of biofilm formation in a bioreactor [416].

8.3.4.2 Effects of combined input parameters

To investigate the combined effect of inputs on the IPB performance, model simulation was carried out with the COD concentration changed from 50 to 400 mg L⁻¹ while the anolyte flow rate varied from 0 to 1.5 mL min⁻¹ at the same time (Figure 8.6). Those two factors actually contribute to the organic loading rate, which is expected to improve current generation to a certain extent. The model predicts that the low flow rate (0.1 to 0.25 mL min⁻¹) and adequate COD input (> 150 mg L⁻¹) is able to maximize current output to 11 mA (Figure 8.6A). However, an extremely small flow rate (< 0.1 mL min⁻¹) will decrease the current output. The daily biomass production rate reaches the peak at the flow rate of 0.1 mL min⁻¹, regardless of the COD input (Figure 8.6B). As explained

previously, a higher flow rate may create a washout effect giving rise to a lower concentration of suspended algal biomass.

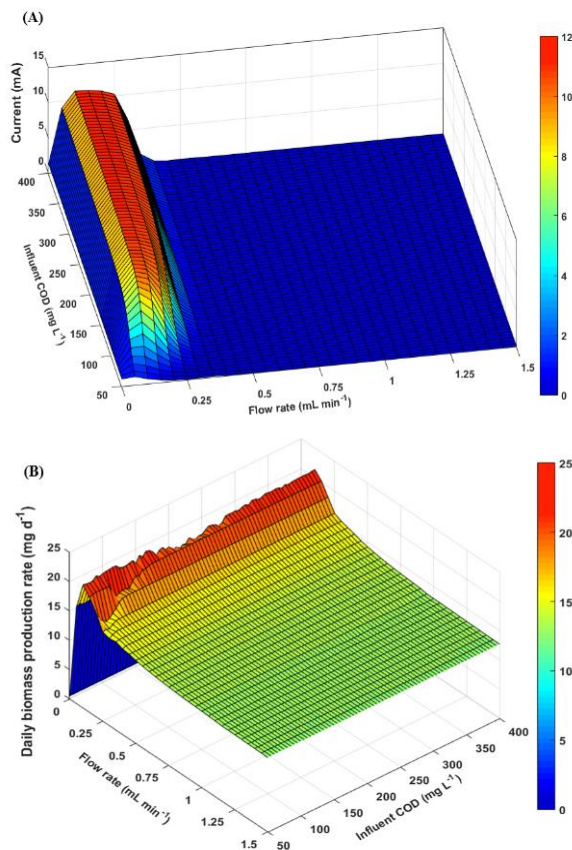


Figure 8.6 Model prediction with variation of two combined factors, the influent COD and the flow rate, for two outputs: (A) current generation; and (B) suspended biomass concentration in the cathode.

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8.3.5 Perspective

The IPB model developed in this study is the first attempt of the kind to integrate the models of MFC and algal bioreactor for simulating the biomass growth, current generation, and nutrient removal. Despite good fitting and small RSME, the present IPB model has some limitations and will need further improvement. The model was constructed based on many assumptions (Table G2) to facilitate the model computation by only capturing the main impacting factors. However, the simplification may have ignored certain factors that can strongly impact the IPB performance. For example, the cathodic pH was assumed as 8.25 in this study; but the actual pH could be varied and this variation will be an important influence factor on the cathode overpotential and nutrient

removal by nitrification and denitrification. The influence of pH change will be considered into IPB model in the future to improve model accuracy and embody the effect of pH on IPB performance. The lack of physical mixing in the cathode did not create a homogenous condition as assumed and this discrepancy might have contributed to the modeling error; this could be improved in the future by either providing mixing or considering heterogeneous factors for the cathode. In addition, the model is relatively insensitive to predict the impact of high concentrations of influent COD, $\text{NH}_4\text{-N}$ and TP because the model construction and validation is derived from the data obtained from low input concentration, limiting the model application for the IPB system to receive high strength wastewater.

Moreover, the model did not consider the factor of algal taxa on IPB performance, and thus the IPB model cannot reflect the role or contribution of specific algal species on current generation, nutrient removal efficiency and biomass production. Actually, the cathode contains mixed population of algae and bacteria, and the synergistic performance of algal-bacterial consortium is important for IPB performance [111]. For example, some algal taxa (e.g., *Chlamydomonas reinhardtii*, *Chlorella vulgaris*) have strong tolerance ability to treat various wastewater and a fast growth rate [417], while bacterial taxa *Rubrimonas cliftonensis* was reported to be capable of nitrate reduction [4]. Studying the working mechanism of these species will help not only the improvement of IPB performance but also the promotion of model functions to give better predication on selecting suitable microbial species.

Improving parameter re-estimation will help to solve some of the above problems. Sensitivity analysis applied in this study is to evaluate the system output by adjusting one parameter while fixing the others. However, more complicated models are expected when actual wastewater is treated in IPB systems, and more parameters are needed to construct a new model. Thus, adjustment of only one parameter at a time may not be workable, because it is difficult to tell whether other parameters are already at the optimized value for best system performance. Advanced statistical strategies or standards are needed to automatically adjust all parameters at the same time to produce the best parameter combination.

Finally, how the developed model can help with scaling up of the IPB system will be of key importance to IPB development. The present model was developed based on the information collected from small-scale IPB system, and an up-scaled system will face challenges such as uniform mixing, light distribution and difficulty in operation controlling. This will require the design and building of larger scale IPB system with simultaneous optimization of the key design factors guided by the model predication.

8.4. Conclusions

A mathematical model has been developed, calibrated, and validated for simulating and understanding the IPB system performance in terms of current generation, biomass growth and nutrient removal under various operational conditions such as influent COD concentration and the anolyte flow rate. The model output satisfactorily describes the experimental observation, and has good prediction to help set operating conditions to optimize the IPB performance. The innovative IPB modeling system is capable of providing an integrated interpretation for the synergy between electrochemical systems and algal growth, even though further efforts are necessary to improve the model applicability and accuracy. The model can become an effective platform to guide the development of IPB systems to better achieve sustainability and green energy recovery.

Chapter 9 Effective Algal Harvesting by Using Mesh Membrane for Enhanced Energy Recovery in an Innovative Integrated Photobioelectrochemical System

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9.1 Introduction

It has been well recognized that wastewater treatment consumes a significant amount of energy [20], for example about 2-3% of the electrical energy in the U.S. [24]. Sustainable wastewater treatment favors recovery of useful resources such as energy and value-added products with simultaneous waste reduction. The energy potential in the dissolved organics in a municipal wastewater is about 4 kWh kg⁻¹ COD_{removed} [418], and recovery of this energy will help offset energy consumption by the treatment process. Energy can be recovered in the form of biogas, bioelectricity, and/or biomass via different treatment methods. In addition to energy recovery, reducing energy consumption will also play a key role in achieving energy-neutral or even positive wastewater treatment. Because the major energy consumption (> 50%) in the existing domestic wastewater treatment comes from aeration [419], development of anaerobic-based treatment or alternative approaches for providing dissolved oxygen will be of great interest.

As an emerging treatment concept, integrated photobioelectrochemical systems (IPB) link microbial fuel cells (MFCs) with algal growth to accomplish low energy consumption (via anaerobic treatment) and energy recovery (both bioelectricity and algal biomass) [71, 79]. An IPB system can take advantage of MFCs to use electrogenic bacteria to simultaneously degrade organic compounds and generate electricity by oxygen reduction reaction [3]. The oxygen reduction reaction occurs on the cathode like regular MFCs to complete the electrical circuit between the anode and the cathode [28]. In the presence of algae, oxygen is supplied to the cathode via photosynthesis, instead of external aeration, and the anode effluent flows into the cathode compartment as a catholyte for nutrient removal (e.g., N, P) by algae, thereby eliminating the need for aeration and resulting in lower energy consumption [3]. In addition, algal biomass can be

potentially used for production of biofuel or other value-added products such as bioplastic [420]. Bioelectricity harvesting from an IPB system is relatively straightforward and can be accomplished by using power management system [116]. Algal biomass, on the other hand, will need much effort to achieve effective collection. Algal biomass could yield 30-50 % weight to produce oil for biodiesel production, which has an energy density of $\sim 41 \text{ MJ kg}^{-1}$ [421]. Many algal species prefer to form biofilm on a solid surface [397, 422, 423], which is usually the cathode electrode of an IPB. The reported methods for algal harvesting from IPB involve manual scraping and filtration of the catholyte [424, 425]. Manual collection of algal biomass in the cathode requires the MFC to be removed out of the cathode compartment and this would create significant challenges for a practical system. Filtration of the catholyte can only harvest the suspended biomass, and the attached biomass must require manual detachment into the liquid phase, which would be difficult in a large-scale system. Therefore, new harvesting methods will be needed which can be potentially used for scaled IPB systems.

To minimize the effect of algal biomass harvesting on the cathode, separating algal growth from the IPB cathode may be considered but algae should still be adjacent the cathode for providing dissolved oxygen and taking up nutrients. Mesh membrane (MM) has been used to harvest algal biomass and may be installed in an IPB to facilitate algal collection. Various types of MM have been examined for their capability of algal attachment, material durability, and cost effectiveness. It was reported that cotton duct could serve as an effective carrier for the algal attachment in a rotating algal biofilm cultivation system [426]. The stainless steel woven mesh with a membrane pore size of $5 \mu\text{m}$ was shown to achieve excellent production of algal biomass [427]. Different lignocellulosic materials including pine sawdust and rice husk have also been investigated as carriers for the algal attachment with reducing the operational cost for the algal harvesting [428]. Synthetic materials such as nylon and polyester were found to have suitable surface texture and physico-chemical properties to obtain the satisfactory biomass productivity [429]. Other materials that were studied for algal growth and attachment included the glass fiber, plain printing paper, filter paper, and cellulose acetate membrane [423, 430]. Those prior efforts indicate a good potential of using MM to collect algal biomass, for example in an IPB system, but this has not been investigated before.

The goal of this study was to design a configuration for IPB that could facilitate algal collection and to formulate a strategy for effective algal biomass harvesting using MM. Two MM materials, made of nylon and polyester of various pore sizes, were initially examined for algal growth and harvesting in an IPB. The polyester-MM was selected for further experiments to understand the effects of algal growth/harvesting periods and harvesting frequencies on the biomass collection and the energy production. Finally, catholyte recirculation, a method for catholyte mixing, was studied and compared to catholyte mixing and constant aeration for overall energy performance.

9.2 Materials and methods

9.2.1 Mesh membrane material selection

In the algal attachment affinity test, six types of MMs made of polyester or nylon with different pore sizes (polyester at 0.11, 0.53 and 4.98 mm, and nylon at 0.11, 0.53 and 5.31 mm) were divided into three experimental groups (Table H1). Two different MM materials with similar pore size (and the same physical size of 15 x 15.2 cm/each) were simultaneously installed in a water tank (15.2 x 30.5 x 20.3 cm) for side-by-side comparison (Figure H1). Illumination was provided by a light at 12 h on/12 h off. The water tank was filled with 6 L algal solution, consisting of deionized water supplemented with chemicals to mimic domestic wastewater according to a previous study [405], and algae inocula (sampled from Duck Pond on Virginia Tech campus, Blacksburg, VA), with an initial algal concentration of 1 g L⁻¹. Each test would last for four cycles with a four-day period of each cycle to ensure sufficient algal attachment [84], though the four-day period was not necessarily an optimal period for achieving the best algal growth. The weight of the harvested biomass in last three cycles (2nd to 4th cycle) was used for comparison of algal attachment. Two polyester MM materials (0.11 and 0.53 mm) were further studied with eight-cycle cultivation to determine the better pore size for the following IPB experiments (Table H1).

9.2.2 Integrated photobioelectrochemical system setup and operation

An IPB system was composed of two tubular MFCs (hydraulically connected in series and installed in the same water tank as that of the MM selection test) (Figure 9.1). Each tubular MFC was made of cation exchange membrane (CEM, Ultrex CMI7000, Membranes International, Inc., Glen Rock, NJ) to separate the anode and the cathode compartments, with an anode working volume of 200 mL (diameter: 4.4 cm; length: 15 cm). The water tank functioned as both the algal

bioreactor and the cathode compartment for the IPB with a liquid volume of 3 L. The anode electrode material was carbon brush, while the cathode electrode was carbon cloth coated with activated carbon (5 mg cm⁻²) surrounding the CEM, prepared according to the previous work [209]. Each tubular MFC was connected with one external resistor of 8.2 Ω to ensure relatively high current generation, and the total current was the sum of two MFCs.

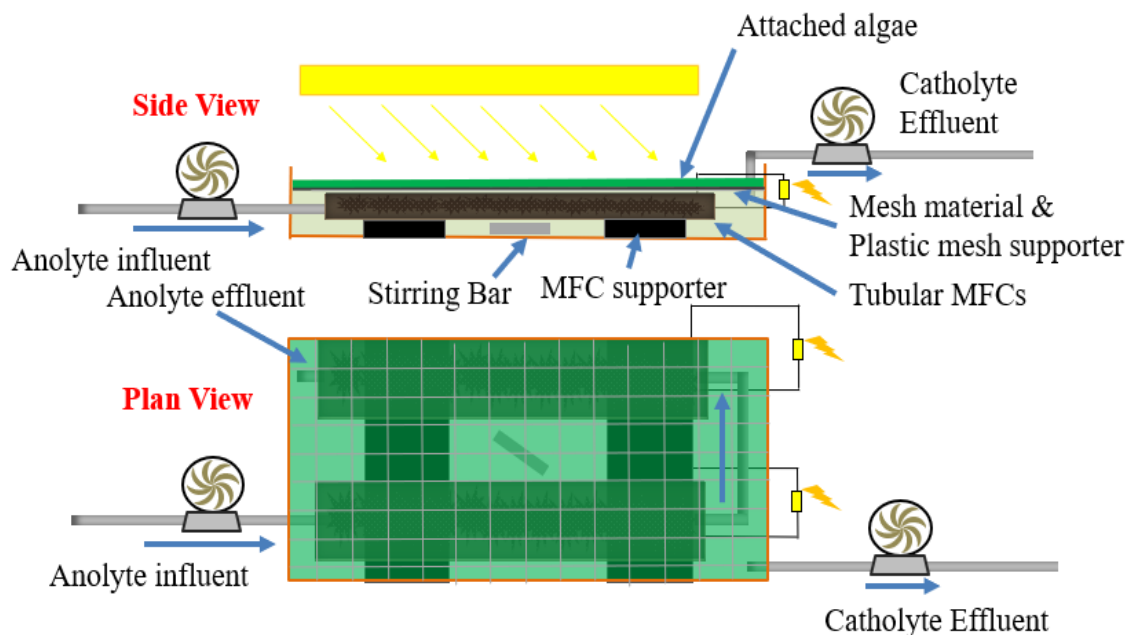


Figure 9.1 Schematic of the IPB system containing mesh membrane for algal growth and harvesting and two tubular MFCs in serially hydraulic connection, using stirring to mix the catholyte.

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Synthetic domestic wastewater was continuously pumped into the IPB anodes at a flow rate of 0.55 mL min⁻¹, resulting in an anodic hydraulic residence time (HRT) of 12 h. It contained (per L DI water): 0.35 g sodium acetate, 0.15 g NH₄Cl, 0.5 g NaCl, 0.015 g MgSO₄, 0.02 g CaCl₂, 0.6 g NaHCO₃, 0.027 g KH₂PO₄, and 1 mL trace element, resulting in a composition of ~ 270 mgL⁻¹ COD, ~ 40 mg L⁻¹ NH₄⁺-N, and ~ 6 mg L⁻¹ total phosphorus (TP). The anolyte effluent was discharged into the water tank as the catholyte, and the catholyte effluent was pumped out of the water tank at the same flow rate as the anolyte (the HRT in the cathode/algal bioreactor was 3.75 days). Two fluorescent bulbs (40 W, 125 V, 4100 K, GE, CT, USA) were installed on the top of the water tank, with light illumination at 12 h off/ 12 h. The catholyte mixing was initially provided by a stirring bar, which was in later experiment replaced by the catholyte recirculation.

9.2.3 Experiments

The experiments consisted of three groups for different purposes (Table H2). Group 1 was to examine the time interval (4, 6 and 8 days) for algal growth/harvesting. Group 2 was to study the partial harvesting (or frequency) by collecting algal biomass formed on a part of MM. To do this, three tests were performed with the same total surface area of MM: the first test was one piece of MM that was the same as that used in Group 1; the second test was two pieces of MM (each was $\frac{1}{2}$ of the size of the total surface area); and the third test was three pieces of MM (each was $\frac{1}{3}$ of the size of the total surface area). In the second and third tests, one (small) piece was removed for algal biomass collection, while the rest were remained in the reactor for future harvesting. The total harvesting time was 6 days in each test; that meant in the first test, the whole piece of MM would be removed for algal biomass collection every 6 days, while in the second and third tests, each (small) piece of MM was removed for algal harvesting every 3 or 2 days (in total, each IPB had 6 days for algal harvesting in each cycle). Group 3 (three pieces of MM in the IPB) was to investigate the method for catholyte mixing, including continuous aeration (50 mL min^{-1} , no algae), stirring (same as that in Groups 1 and 2), and catholyte recirculation. To perform catholyte recirculation, the stirring was removed and the catholyte was returned to the top of MM by a pump at 50 mL min^{-1} (Figure H2).

9.2.4 Measurement and calculations

For the MM material selection, the attached algae on the MMs were scraped off after one cycle and measured for weight. In the IPB experiment, the voltage of the IPB was recorded every 5 min by a digital multimeter (2700, Keithley Instruments, Inc., Cleveland, OH). The concentrations of soluble COD, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and TP were measured by using a DR/890 colorimeter (Hach Company, Loveland, CO, USA). The dissolved oxygen (DO) in the catholyte was measured by using a DO meter (Hanna Instrument, Woonsocket, RI, USA). The total Coulomb (TC) were calculated by integrating current over time in an operational cycle. The removal efficiencies (RE, %) of COD, $\text{NH}_4^+\text{-N}$ and TP were also calculated. The algal biomass on the MM was scraped off and dried in a muffle oven for one day (50°C) to obtain the dry weight, determined as harvested algal biomass (HAB, g). In contrast, non-harvested algal biomass (NHAB, g) was defined as the algal biomass attached on the wall of water tank and the cathode electrode of the MFCs, and suspended biomass in the catholyte.

Surface biomass productivity (SBP, g m⁻² day⁻¹) was used to represent the algal growth rate on the MM [429], and determined as SBP = HAB/surface area of MM, which was 0.0392 m² in this study. Actual algal harvesting efficiency (AAHE, %) was calculated according to Eq. 9.1:

$$AAHE = \frac{HAB}{HAB+NHAB} * 100\% \quad (\text{Eq. 9.1})$$

where HAB represents the total HAB (g) from MM, and NHAB represents the total algal biomass (g) from other sources (e.g., wall surfaces of algal tank).

The total energy generation per day (EG, kJ day⁻¹) was estimated using Eq. 9.2, including both electricity generation (E_e , kJ day⁻¹) and harvested algal biomass (E_a , kJ day⁻¹):

$$EG = E_e + E_a \quad (\text{Eq. 9.2})$$

The energy generation from algal biomass (E_a , kJ day⁻¹) was estimated according to Eq. 9.3:

$$E_a = \frac{HAB * \eta_1 * \eta_2 * 3.78 * 10^4}{t} \quad (\text{Eq. 9.3})$$

where η_1 and η_2 represent the converting efficiency from harvested algal biomass to biodiesel (η_1 assumed as 0.4) and from the biodiesel to electricity (η_2 assumed as 0.3) [3, 432], respectively; the number “3.78 * 10⁴” represents the energy content (kJ) of harvested algal biomass per gram of HAB; and t represents the total time period (day) for each test (e.g., $t = 18, 12$ and 24 days for experimental group # 1, Table H2).

The energy consumption (EC) included the solution pumping, catholyte recirculation (in later experiment), stirring for catholyte mixing (in early experiment), and/or aeration (in the test that did not have algae inoculation). The energy consumption by the solution pumping (E_p , kJ day⁻¹) was calculated based on the following equation (Eq. 9.4) [244]:

$$E_p = (2 * Q_1 * E_1 + Q_2 * E_2) * \frac{86.4 * r}{6 * 10^7} \quad (\text{Eq. 9.4})$$

where Q_1 represents the flow rates of anolyte influent and catholyte effluent ($Q_1 = 0.55$ mL min⁻¹); Q_2 represents the flow rate of the catholyte recirculation rate effluent ($Q_2 = 50$ mL min⁻¹ for group 7; $Q_2 = 0$ mL min⁻¹ for experimental groups without catholyte recirculation); E_1 and E_2 represent

the hydraulic pressure heads of two pumps (m) for the anolyte influent/catholyte effluent ($E_1 = 0.016$ m in this study) and the catholyte recirculation ($E_2 = 0.025$ m in this study), respectively; and r is 9800 N m^{-3} . The energy consumption by the stirring (kJ day^{-1}) was assumed as 0.008 W with 400 rpm (0.69 kJ day^{-1}) according to the power consumption of turbine impeller in a 2 L stirring miniature bioreactor [433]. The energy consumption of aeration (in group 3) was estimated as $160.7 \text{ kJ day}^{-1}$ with the aeration rate at 50 mL min^{-1} (0.62 kWh m^{-3} air based on a previous reference [434]).

The net energy production (NEP, kJ day^{-1}) is the difference between energy generation and consumption. A negative NEP means that energy input is required, while a positive NEP indicates net energy output.

9.3 Results and discussion

9.3.1 Selection of optimal mesh membrane material

The selection of optimal MM material was performed by examining algal growth on two types of MM materials with three different pore sizes each (Table H1). The results showed that the 1st cycle generally had very low SBP, because of the initiation for the algal attachment, and the 3rd and 4th cycles exhibited close results (Figure H3). This confirmed the previous findings that the SBP from the regrowth culture in later cycles would be higher than the initial growth [429, 435]. The MM comparison was based on the average SBPs from 2nd to 4th cycles. The MM made of polyester with a pore size of 0.11 mm and 0.53 mm had the higher SBPs (2.41 ± 0.41 and $2.82 \pm 0.33 \text{ g m}^{-2} \text{ day}^{-1}$, respectively) than that of other MMs ($p < 0.05$, two-tailed t-test) (Figure 9.2), likely benefited from better surface texture of the polyester material for algal attachment than nylon material in this study [429, 436]. However, the SBP comparison only based on 2nd to 4th cycles was not sufficient to determine the pore size (0.11 mm and 0.53 mm) for the algal attachment, due to the possible cultivation instability in the initial cycles.

Next, a relatively long-term operation (8 cycles for 32 days, Table H1) was performed to compare the MM made of polyester with two pore sizes, 0.11 and 0.53 mm , and the data from the last four cycles (5th to 8th cycles) were used for analysis. It was found that the SBP of the polyester with 0.53 mm had $4.13 \pm 0.21 \text{ g m}^{-2} \text{ day}^{-1}$, significantly higher than that with 0.11 mm ($3.60 \pm 0.27 \text{ g}$

$\text{m}^{-2} \text{ day}^{-1}$) (inset of Figure 9.2, $p < 0.05$). This result was unexpected, because the attached algal attachment was supposed to increase with decreasing pore size that had better retention [429, 437]. The surface texture of 0.53-mm MM might have helped with algal attachment, because that the single algal cells would easily accumulate to form the cluster with much bigger size (possibly greater than 0.11 mm pore size MM in this study), which could make algae difficult to retain on the MM with smaller pore size, but a very large pore size would also decrease algal attachment by allowing algal cells to pass through (Figure H3). This was supported by a previous study that showed the best algal attachment with 0.5-mm mesh openings [429]. Therefore, the polyester MM with the pore size of 0.53 mm was chosen for the following experiments. It should be noted that the surface properties of the MM need to be studied by using techniques such as contact angle (hydrophobicity/hydrophilicity) and SEM/AFM (roughness), and chemical properties of the MM should be evaluated in order to determine any possible linkage of surface with algae. All of these will provide new directions in research of IPB systems.

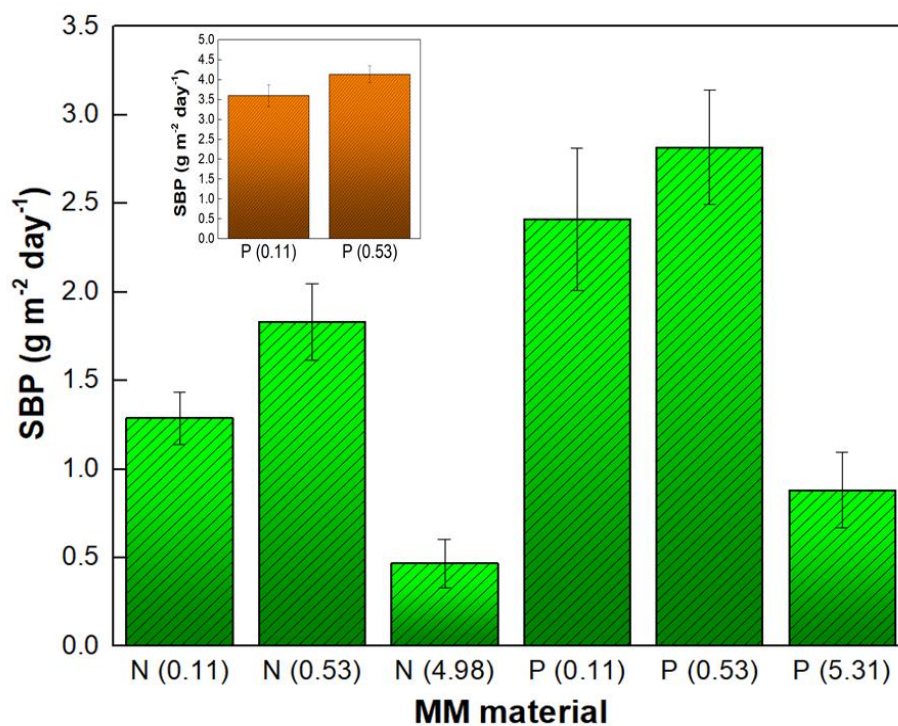


Figure 9.2 Average surface biomass productivity (SBP, $\text{g m}^{-2} \text{ day}^{-1}$) in three cycles (2nd to 4th cycle) for two different MM materials (“N” represents nylon; “P” represents polyester) and different pore sizes. Inset: comparison of SBP (5th to 8th cycles under stable algal growth) for the polyester MM with two pore sizes, 0.11 and 0.53 mm.

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9.3.2 Effects of algal growth/harvesting period (Group 1)

Both the algal attachment and harvesting on the MM was strongly relevant to the algal growth, the period before harvesting. Three growth/harvesting periods, 4, 6 and 8 days, were examined (Group 1, Table H2). The growth/harvesting period changed DO and thus affected current generation (Figure 9.3A and B). It was observed that the peak current increased from 1.50 mA on the 1st day to 3.68 mA at the end of 6th day for the growth/harvesting period of 6 days, with the peak DO concentration increasing from 2.80 (1st day) 5.91 mg L⁻¹ (6th day). The increased DO concentration was a result of more algal accumulation on the MM and increased oxygen production rate over time. The IPB with the 8-day growth/harvesting period had a total Coulomb production of 152.7 ± 36.1 C day⁻¹, significantly higher than that of the 4-day growth/harvesting period (92.5 ± 31.6 C day⁻¹) ($p < 0.05$), but not significantly different from that of the 6-day growth/harvesting period (121.3 ± 37.0 C day⁻¹) ($p > 0.05$). Regardless of the algal growth/harvesting periods, the IPB achieved comparable treatment performance, in terms of the removal efficiencies of COD, NH₄⁺-N, and TP (Figure 9.3C). The COD removal efficiency was 61.6 ± 0.83 %, 62.3 ± 3.42 %, and 60.4 ± 2.38 %, for the growth period of 4, 6, and 8 days, respectively ($p < 0.05$). In general, the IPB could remove 60-70% of NH₄⁺-N and 25-40% of TP under all algal growth periods.

The IPB operated under the algal growth/harvesting period of 6 days had a SBP of 0.88 g m⁻² day⁻¹, greater than 0.78 and 0.83 g m⁻² day⁻¹ of 4 and 8 days, respectively (Figure 9.3D). The lower productivity of the 8-day growth/harvesting period was possibly because that the formation of thick algal biofilm might have inhibited the light penetration and the transfer of carbon dioxide from the air for algal growth [426]. In addition, the sloughing effect against the algal growth might be another reason to inhibit the algal growth with a longer growth/harvesting period [438]. For the shorter growth/harvesting period of 4 days, frequent harvesting would keep the algal growth constantly at a lag phase, with limited time for the acclimatization and growth before harvesting [426]. Thus, the optimized algal growth period would be at 6 days in the present study. An appropriate algal growth/harvesting period could also improve energy generation, which included both electricity and algal biomass. The energy generation of the 6-day growth/harvesting period was 0.157 ± 0.001 kJ day⁻¹, statistically higher than 0.148 ± 0.002 kJ day⁻¹ of 8 days ($p < 0.05$, Figure 9.3D), and the major difference in energy generation was due to different algal biomass production. More algal biomass with the 6-day growth/harvesting period also benefited from its

higher harvesting efficiency of 58.4 % (Figure H4). The lower harvesting efficiency of the 8-day growth/harvesting period (53.1%) was possibly attributed to the cell shading and sloughing effect due to stronger susceptibility with thicker algal biofilm, and thus re-grown algae was easily to be washed off MM under a turbulent condition [438, 439]. In summary, an algal growth/harvesting period of 6 days was determined to be optimal and used in the following experiments.

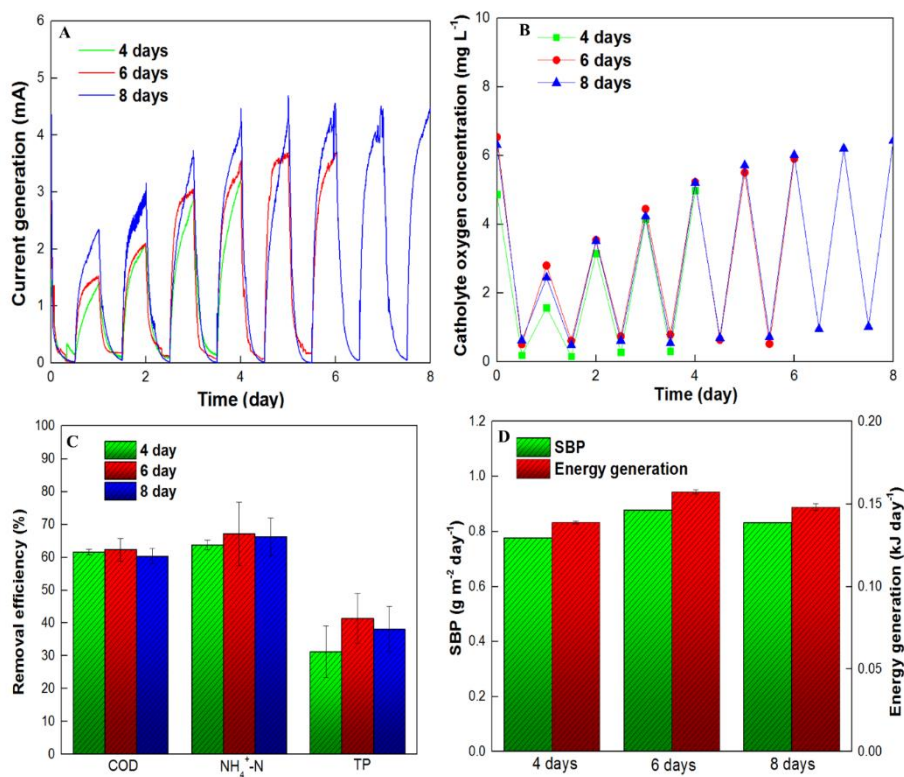


Figure 9.3 The IPB performance affected by the algal growth/harvesting periods, 4, 6 and 8 days (Group 1): A) current profile; B) catholyte oxygen concentration; C) removal efficiency of COD, NH₄⁺-N and TP; and D) SBP and daily energy generation (kJ day⁻¹).

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9.3.3 Effect of harvesting frequency in a cycle (Group 2)

Because of the benefit of residue algal biomass to continuing growth (for next harvesting cycle), it would be of interest to investigate whether the biomass should be collected completely at one time or partially with multiple collections within a cycle. To do this, three tests were performed with one, two, and three pieces of MM, each of them had the same total surface area, and each test had the same total growth/harvesting period of 6 days. It meant that in the case two pieces of MM,

each would be removed for algal biomass harvesting every three days, and in the case of three pieces of MM, each would be harvested every two days (Table H2). The peak current generations at the end of one cycle for all the harvesting frequencies at 1, 2 and 3 time/cycle days were similar in the range of 3.5 to 3.7 mA, indicating the limited impact of changing harvesting frequencies on the current generation after enough time for the algal cultivation on MM (Figure 9.4A). The total Coulomb production of three tests were not significantly different, at $141.3 \pm 13.4 \text{ C day}^{-1}$, $124.9 \pm 27.2 \text{ C day}^{-1}$ and $121.3 \pm 37.0 \text{ C day}^{-1}$ with the harvesting frequencies at 2, 3, and 1 time/cycle days, respectively ($p > 0.05$). Thus, the difference of the harvesting frequencies did not significantly affect the current generation. In addition, the IPB did not behave differently in terms of the organic and nutrient removal for all harvesting frequencies, and achieved the removal efficiency of about 60-65 %, 60-70 % and 30-50 % for COD, $\text{NH}_4^+\text{-N}$, and TP, respectively ($p > 0.05$, Figure 9.4B).

It was found that partial harvesting (multiple collections in a cycle) could improve algal biomass production and thus energy generation. The SBP of the third test (with three pieces of MM) was the highest at $1.14 \text{ g m}^{-2} \text{ day}^{-1}$, compared to that of the one-piece MM test ($0.88 \text{ m}^{-2} \text{ day}^{-1}$) and the two-piece MM test ($1.04 \text{ g m}^{-2} \text{ day}^{-1}$) (Figure 9.4C), while the algal harvesting efficiency was comparable among the three tests (Figure H5). This high SBP in the third test resulted in the most energy generation of $0.205 \pm 0.009 \text{ kJ day}^{-1}$, significantly higher than that in the other two tests ($p < 0.05$, Figure 9.4C). However, more frequent harvesting would also lead to more algal loss, due to frequent manual scraping. The total amount of the uncollected algal biomass in the third test was $0.0201 \text{ g day}^{-1}$, greater than that of the one-piece MM test ($0.0163 \text{ g day}^{-1}$) and two-piece MM test ($0.0180 \text{ g day}^{-1}$) (Figure H5). In general, algal harvesting based on the three-piece MM approach could benefit algal growth and collection, and was used in the following experiments.

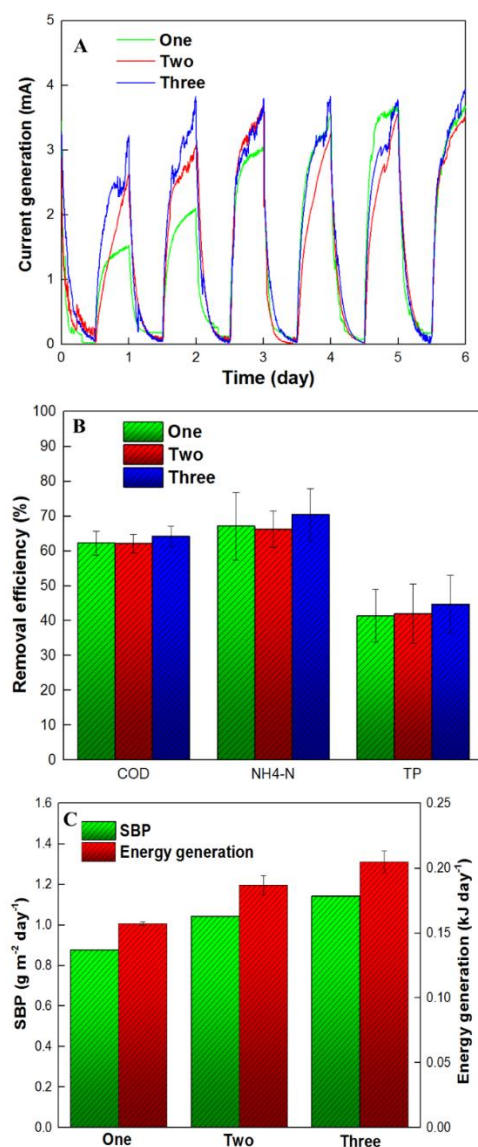


Figure 9.4 The IPB performance affected by harvesting frequency: (Group 2): A) current generation; B) removal efficiency of COD, NH₄⁺-N and TP; and C) SBP and daily energy generation. Note: “one, two, three” represent three tests in this group that contained different number of MM pieces with the same total surface area in each test.

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9.3.4 Effects of the catholyte mixing method (Group 3)

Catholyte mixing is critically important to provide sufficient mass transfer of both DO (to the cathode for reduction reaction) and nutrients (to algae for growth). However, the use of stirring to provide mixing is not practical. In a treatment system, mixing is usually provided by either

mechanical agitation, aeration, or hydraulic recirculation. In this experiment, hydraulic recirculation was employed by recirculating the catholyte; for comparison, constant aeration of air in the absence of algae was also conducted (Group 3, Table H2).

The current generation of three operational conditions was shown in Figure 9.5A. The system with constant aeration exhibited average current output of 3.22 mA and the average current density based on the anode volume was 8.05 A m^{-3} . For the IPB system, the use of algae to provide DO resulted in day/night variation affected by photosynthesis, and thus the average current densities (including current generation at night) under the stirring and the catholyte recirculation conditions were only 4.93 A m^{-3} and 5.11 A m^{-3} (anode volume) during 5th to 8th cycles, respectively. The total Coulomb production and the Coulombic efficiency with the aeration was $276.6 \pm 1.3 \text{ C day}^{-1}$ and $20.9 \pm 0.6 \%$, much higher than those by either the stirring ($169.4 \pm 39.9 \text{ C day}^{-1}$ and $12.1 \pm 2.5 \%$) or the catholyte recirculation ($176.0 \pm 21.8 \text{ C day}^{-1}$ and $12.6 \pm 1.3 \%$) ($p < 0.05$). Such difference was due to DO supply, which influences cathodic oxygen reduction reaction and thus the current generation. The IPB performance of COD and $\text{NH}_4^+\text{-N}$ removal was comparable to that with constant aeration (Figure 9.5B, $p > 0.05$). However, the treatment efficiency of TN and TP was significant enhanced in the presence of algae, at $46.04 \pm 3.15 \%$ (TN) and $43.95 \pm 4.53 \%$ (TP) with the stirring, or $37.53 \pm 5.14 \%$ (TN) and $42.36 \pm 2.69 \%$ (TP) with the catholyte recirculation, higher than $25.33 \pm 2.91 \%$ (TN) and $7.41 \pm 5.69 \%$ (TP) with constant aeration ($p < 0.05$). The introduction of algae for their attachment on the mesh membrane would also bring various bacterial species into the IPB cathode (in both solution and cathodic biofilm), especially after a long-term operation [440]. In addition, the oxygen concentration difference between the daytime and night, and the oxygen gradient between cathodic biofilm and the solution would colonize different anaerobic and aerobic microorganisms with various metabolic pathways, some of which could improve the nutrient removal, such as the nitrification and denitrification [441]. Thus, the higher TN and TP removal rates in the IPB demonstrated the importance of algae in nutrient removal in the proposed system.

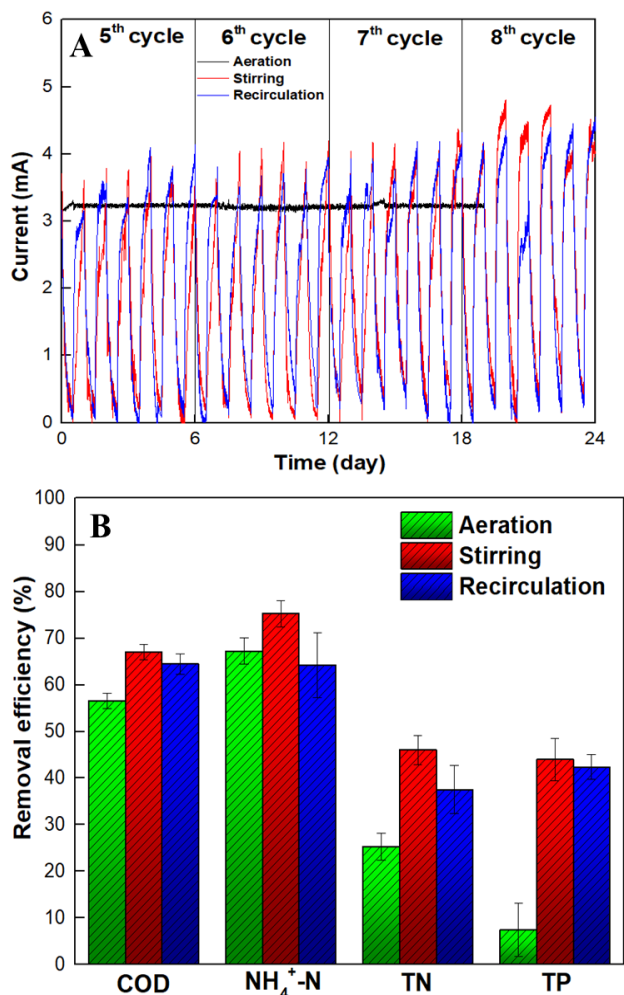


Figure 9.5 The IPB performance under three catholyte mixing/DO supply conditions (Group 3): A) current generation; B) removal efficiency of COD, NH₄⁺-N, TN (including NH₄⁺-N and NO₃⁻-N) and TP.

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The SBP trend was similar to the trend of the cathodic oxygen concentration for both operating conditions (Figures 9.6A and B; note: the aeration-system did not have algal growth), but the IPB with the stirring produced a lightly higher SBP of $1.62 \pm 0.06 \text{ g m}^{-2} \text{ day}^{-1}$ than that using the catholyte recirculation ($1.46 \pm 0.06 \text{ g m}^{-2} \text{ day}^{-1}$, $p < 0.05$), based on the algal harvesting in 5th to 8th cycles (Figure 9.6A), likely related to the flushing effect of the catholyte recirculation that could wash off the algae from the MM. This washing-off effect could also be the reason of a lower cathodic oxygen concentration in the early stage of the operation (1st to 4th cycles with slower algal biofilm development) under the condition of the catholyte recirculation; eventually, both conditions had similar cathodic oxygen concentrations ($6.05 \pm 0.78 \text{ mg L}^{-1}$ with the catholyte

recirculation vs. $6.41 \pm 0.31 \text{ mg L}^{-1}$ with the stirring, Figure 9.6B, $p > 0.05$). The methods to improve the SBP warrant future investigation, and the optimization of the bicarbonate concentration in the cathode is promising because it affects the photosynthetic strength and the oxygen concentration [442]. Also, the HRT of cathode and catholyte flow rate would other directions deserved to study in the future.

The energy performance of three catholyte mixing conditions from the relatively stable period (last 4 cycles) was shown in Figure 9.6C. Despite higher total Coulomb production, the aerated MFC had energy generation of only $0.004 \pm 0.001 \text{ kJ day}^{-1}$, much lower than that under the stirring condition ($0.292 \pm 0.017 \text{ kJ day}^{-1}$) or the catholyte recirculation ($0.263 \pm 0.025 \text{ kJ day}^{-1}$), because of the lack of algal biomass production. In addition, the energy consumption of the aerated MFC was $-160.7 \text{ kJ day}^{-1}$, several orders of magnitudes higher than that the IPB system under the stirring condition ($-0.691 \text{ kJ day}^{-1}$) or the catholyte recirculation ($-0.036 \text{ kJ day}^{-1}$). As a result, the aerated MFC had the most negative NEP of $-160.695 \pm 0.001 \text{ kJ day}^{-1}$ (or the largest net energy demand). For the IPB system under two catholyte mixing conditions, the catholyte recirculation resulted in a positive NEP of $0.227 \pm 0.025 \text{ kJ day}^{-1}$, while the stirring still led to a negative NEP of $-0.411 \pm 0.023 \text{ kJ day}^{-1}$ due to its higher energy consumption than production. Compared with other IPB systems with the tubular MFCs (Table H3), the algal productivity normalized to per volume of treated wastewater of this study under the catholyte recirculation was 72.3 g m^{-3} (treated wastewater), which was comparable or even greater than that of other studies. Therefore, the catholyte recirculation was proved to be an effective mixing method with the best overall energy performance.

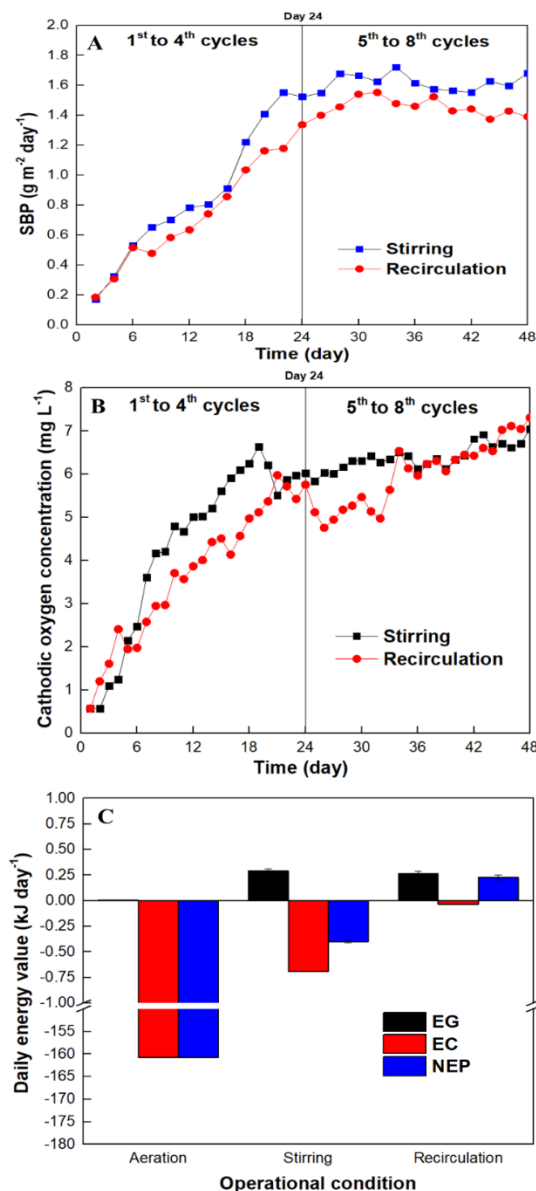


Figure 9.6 The energy performance of the IPB system under three catholyte mixing/DO supply conditions: A) SBP (g m⁻² day⁻¹) under the conditions of stirring and catholyte recirculation; B) cathodic oxygen concentration under the conditions of stirring and catholyte recirculation; and C) energy generation (EG), consumption (EC), and net energy production (NEP) under three conditions (data from the 5th to 8th cycles).

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9.4 Conclusions

This study has demonstrated a new method for algal collection based on mesh membrane in a newly designed IPB system that had algal growth physically separated from the cathode electrode

to facilitate harvesting. Both MM material and pore size were experimentally determined for optimal algal growth. The key factors such as algal growth/harvesting period and harvesting frequency were examined. It was shown that the catholyte recirculation was an effective approach for catholyte mixing with a theoretically positive energy output from the IPB. Those results would encourage further efforts of developing IPB systems for sustainable bioenergy recovery from wastewater treatment.

Chapter 10 Construction and long-term evaluation of scale-up integrated photobioelectrochemical system (60 L) in wastewater treatment and energy generation

10.1 Introduction

Global sustainability is the ultimate goal for the human development in the future [5], and to achieve the goal, the energy conservation is a must to focus on and spare no effort to achieve. In the angle of the water supply and quality protection, the wastewater treatment is a significant procedure to remove the organic contaminant and nutrient, to complete the water purification and guarantee the water quality for the people to drink. The conventional treatment procedure is to use the activated sludge with the mechanical aeration to provide significant oxygen to remove organic and nutrients, but this procedures consumes a significant amount of energy [20, 443]. It was reported that about 2-3% of the electrical energy was consumed by the wastewater treatment in the U.S. [24], which would be a barrier for the global sustainability in the wastewater treatment. Thus, the specific technologies need to be discovered to remediate the energy consumption.

One strategy is to make advantage of the ability of the algae to directly take up the organic and nutrient in the heterotrophic growth mode, to directly remove the organic and nutrient in the wastewater [444]. Also, the wastewater is actually a low-cost and excellent medium for the microalgal growth [445], and the algae are happy to directly use the wastewater to grow. The grown algae biomass can even be converted to the biofuel to recover the energy from the wastewater [86]. Also, another technology, microbial fuel cell (MFC), has the similar function to utilize the electricigens inside the anaerobic organic to convert the organic to the electrons, which could be directly captured as the electric energy in the closed circuit designed by the MFC [28]. The combination of these two technologies would be powerful integrated system to achieve the sustainable wastewater treatment, and thus the integrated system, combining the MFC and algal bioreactor together as the integrated photobioelectrochemical system (IPB), was born at the right moment to benefit the global sustainability.

The synergistic relationship between the MFC and algal bioreactor enables the IPB system to be consistently working with high efficiency on the energy recovery. The MFC cathode needs the oxygen reduction reaction (ORR) to complete the closed circuit, and the required oxygen can be

supplied by the algal photosynthesis in the cathode, replacing the conventional mechanical aeration [3]. Also, the MFC effluent with the C, N and P supply can be an excellent medium for the cathodic algae to grow, and the algal ability to take the nutrient can be a supplemental treatment stage to improve the ability of MFC on the nutrient removal [70]. The synergistic relationship enables the IPB with the powerful function to treat the wastewater with high energy recovery efficiency. For example, it was reported that one IPB system at the scale of 2 L fed with the real wastewater at the organic loading rate at $0.87 \text{ kg COD m}^{-3} \text{ d}^{-1}$, could have the highest energy generation at 0.089 kWh m^{-3} [4]. Also, another IPB study with the cathode volume at 3 L achieved the net energy production at $0.227 \text{ kJ day}^{-1}$, further proving that the IPB system was competent to efficiently recover the energy from the wastewater [431].

However, most of the IPB systems were only constructed as the bench scale, and the feasibility and practical application of the system in the real wastewater treatment has not been tested yet. The actual application would depend on the performance of the scale-up IPB system, and more information is needed to understand the application value of the IPB system in the real wastewater treatment. Based on the current condition to have larger capacity to treat the wastewater to recover, most algae were cultivated in the open pond system due to its easy characteristics to combine with the wastewater treatment process, high sunlight utilization efficiency and low capital cost [446, 447], and thus the scale-up IPB system can be constructed in the form of open pond, with the tubular MFCs placed into the open pond to set up the IPB system [79]. Thus, this study was to set up 60 L scale-up IPB system fed with the real wastewater from Virginia Tech, to evaluate the long-term performance of the IPB system under seasonal variation, and gave more information about the IPB construction and operation.

10.2 Methods and Materials

10.2.1 IPB setup and operation

A scale-up open pond IPB system was constructed of word boards, with the dimensions as 100 cm x 60 cm x 40 cm, and the total capacity was about 240 L (Figure 10.1A). There were four channels to separate open pond, with totally six tubular MFCs in the channels. The 1st and 4th channels had one U-type tubular MFC for each channel, while 2nd and 3rd chamber had two tubular MFCs for each channel. Each tubular MFC was mainly constructed by the cation exchange membrane (CEM,

UltexCMI7000, Membranes International, Inc., GlenRock, NJ, USA), with the anode volume of ~ 2.5 L, and the total anode volume was 15 L. All six U-type tubular MFCs were hydraulic connected in series. Two carbon brushes (70 cm long) were applied anode material for each U-type tubular MFC [404], while the cathode material was the carbon cloth with surface area at 1900 cm^2 for each U-type tubular MFC (PANEX 30PW03, Zoltek Corporation, St. Louis, MO, US), coated with activated carbon (5 mg cm^{-2}), using the same treatment procedures as the previous work [209]. The cathode volume of each channel was around 15 L, and the total cathode volume was about 60 L. On the top of the scale-up reactor, a transparent Plexiglass sheet was placed, to avoid the intrusion of external objects (e.g., leaves, rocks, rains) into the IPB systems to make huge interruption on the IPB performance.

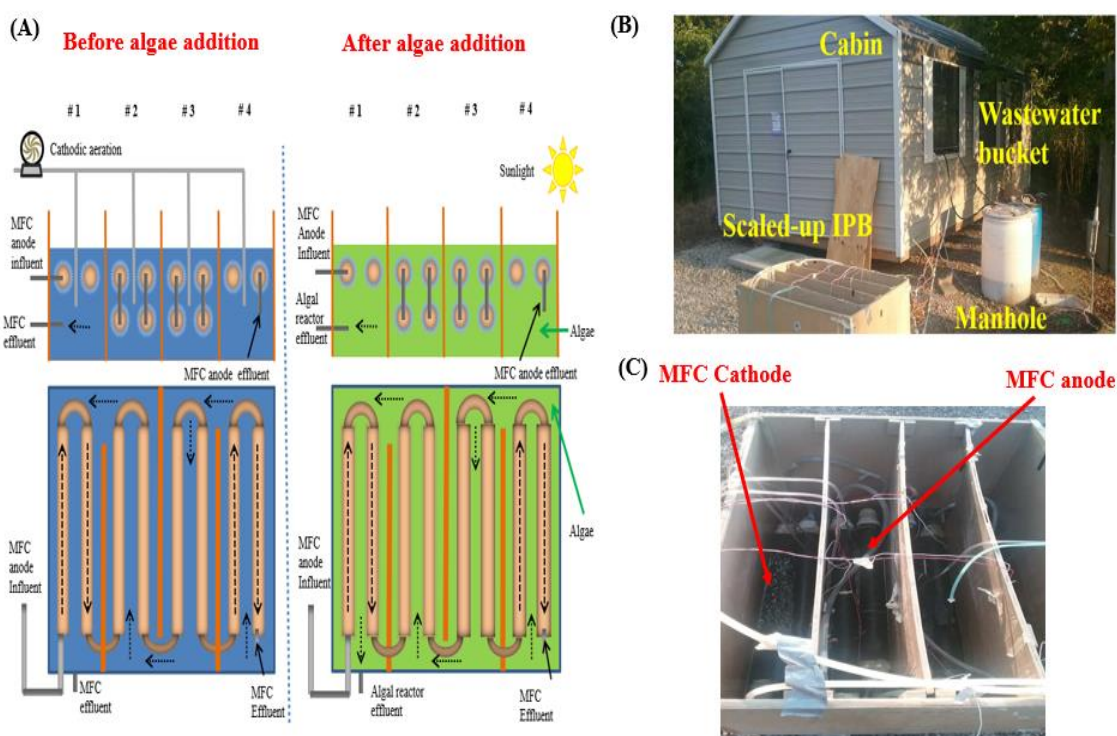


Figure 10.1 Schematic of open pond scale-up IPB system: A) structure schematic of 4-channel scale-up IPB system under aeration and after algae addition; B) actual picture of the entire overall setup for the IPB system; C) actual picture of the internal structure of the IPB system.

The real wastewater was taken from the manhole beside the cabin in Virginia Tech (Figure 10.1B). The wastewater was first pumped into the storage tank (200 L), and then a peristaltic pump was applied to continuously feed the wastewater into the anode at the flow rate of 20.8 mL min^{-1} (Figure

10.1A), to guarantee the hydraulic residence time (HRT) of the entire anode as 12h. The anolyte was flowing from 1st channel to 4th channel, and then the anolyte effluent was flowing back to the cathode of 1st channel. The catholyte also flowed from 1st to 4th channel, and finally another pump was used to take the catholyte effluent out at the suitable flow rate to maintain the same catholyte height level all the time, to guarantee the cathode HRT at 2 days. All the anodes were submerged under the catholytes (Figure 10.1C). The anode and cathode of each U-type tubular MFC in each channel were connected with the external resistance with 1 and 100 Ω , to evaluate the IPB performance with various current generation (more detailed experiment design shown in Table 10.1). The 2nd and 3rd channel with two U-type tubular MFCs were to respectively combine the anode and cathode of each MFC together, and use one external resistor to connect.

At the initial period of the IPB operation, mechanical aeration was given to each channel to start the system as the conventional open-pond MFC system (Figure 10.1C). Two bars of aeration stones (length: 20cm, PetSmart, Phoenix, AZ, USA) were submerged under the catholyte to diffuse the aerated bubbles into the cathode to supply oxygen as the electron acceptor. The aeration speed was at about 800 mL min⁻¹ with two aeration stones (Figure 10.1C). After sufficient time to cultivate the MFC with the mature performance, to gradually convert the system to scale-up IPB system, one aeration stone of each channel was taken out and left only one aeration stone, and thus the aeration speed was halved down to about 400 mL min⁻¹. At the same time, the mixed algae mixture (taken from Duck Pond in Virginia Tech) previously cultivating in the algal bioreactor were added into the cathodes, to achieve OD at about 0.01 for all channels (suspended algal concentration at 1.7 mg L⁻¹) [431]. The reason to still place one aeration stone to give certain extent of the mechanic aeration was to expect to maintain the MFC performance during the algal growth period before the algae could independently supported the MFC performance by photosynthesis. Finally, the aeration stone was taken out and the scale-up IPB system without the mechanical aeration was finally constructed. However, with the temperature decreasing in winter, the algae lost its function under the cold temperature, and thus one aeration stone was placed back into each channel of the IPB to purposely supply the oxygen and restore the MFC performance. The IPB system was operated for nearly four months, from August to December, 2018, and the summary of the experimental design was shown in Table 10.1. The influent in the storage tank, and the

effluent samples of anode and cathode of each channel were taken once every three days for the measurement of the water parameters (described in next section).

Table 10.1 Experimental design of the outdoor scale-up IPB system during four months.

Group #	Time period	Aeration stones #	External resistance (Ω)	Algae added in cathode
1	Aug 15 th to Aug 27 th	Two	100	No
2	Aug 27 th to Sep 27 th	Two	1	No
3	Sep 27 th to Oct 24 th	One	1	Added
4	Oct 24 th to Nov 15 th	None	1	Added
5	Nov 15 th to Dec 5 th	One	1	Added

10.2.2 Measurement and calculation

The voltage of each channel was recorded every 20 min by a digital multimeter (2700, Keithley Instruments, Inc., Cleveland, OH). The concentrations of the soluble COD, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, total phosphorus (TP) of each channel were measured by using a DR/890 colorimeter (Hach Company, Loveland, CO, USA). The dissolved oxygen (DO) of each channel was also measured by DO meter (Hanna Instrument, Woonsocket, RI, USA). The total Coulombs (TC) and Coulomb efficiencies (CE) of each channel were calculated by integrating current over time in a certain period versus the theoretical Coulomb production for the total COD degradation. The removal efficiencies (RE, %) of COD, total nitrogen (TP) and TP were also calculated for each channel. The influent parameters of pH, conductivity, COD, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and TP were shown in Table 10.2. It should be noted that the high TP concentration in the effluent was possibly due to massive discharge of grey wastewater with high TP (even able to reach 120-180 mg L⁻¹) from the daily activities of the university members (e.g., students) [448, 449], and the evaluation for the IPB system to treat the high TP wastewater could be made. The optical density (OD) of the catholyte sample of each channel was also measured at 680 nm, to indicate the algal concentration and growth condition in the open pond IPB system. The equation to calculate suspended algal concentration was based on the standard curve of the previous study [405]. The local ambient temperature (Blacksburg, VA) along the experiment was derived from the website (www.wunderground.com), to evaluate the impact of the temperature on the system performance.

Table 10.2 The influent parameters into the scale-up IPB under each experimental group.

Group #	pH	Conductivity (mS cm ⁻¹)	Soluble COD (mg L ⁻¹)	NH ₄ ⁺ -N (mg L ⁻¹)	NO ₃ ⁻ -N (mg L ⁻¹)	TN (mg L ⁻¹)	TP (mg L ⁻¹)
1	6.18 ± 0.05	0.765 ± 0.012	155.5 ± 8.5	38.5 ± 2.5	0.5 ± 0.3	39.0 ± 2.5	52.0 ± 3.5
2	6.30 ± 0.08	0.788 ± 0.009	160.2 ± 5.6	41.0 ± 3.2	1.0 ± 0.2	42.0 ± 3.2	61.0 ± 4.0
3	6.52 ± 0.07	0.842 ± 0.009	202.2 ± 10.5	48.8 ± 1.5	4.3 ± 1.2	52.8 ± 1.9	70.0 ± 6.5
4	6.38 ± 0.07	0.750 ± 0.005	240.5 ± 9.8	50.3 ± 2.2	14.3 ± 2.3	64.3 ± 3.2	101.5 ± 12.3
5	6.46 ± 0.08	0.752 ± 0.007	219.8 ± 6.3	48.5 ± 1.2	7.3 ± 1.7	55.5 ± 2.1	88.5 ± 8.3

10.3 Result and discussion

10.3.1 Electric current and energy generation

The real wastewater stored in the bucket was fed into and flowed in the anode and cathode sequentially, and the IPB system was initially started as the conventional open-pond MFC system. The electricity was generated in each channel, and collected to evaluate the system performance, under five experimental groups. In the 1st group, due to the high resistance connection for the tubular MFCs of each channel (100 Ω), the current generation was low, while the current greatly increased after changing the external resistance to 1 ohm in the 2nd experimental group (Figure 10.2A). Setting the 2nd channel as the representative to describe the results due to its maximum current generation among all channels. The maximum current generation during daytime reached the range at 15-20 mA in the later period of the 2nd group, much higher than the current generation at 2-3 mA in the 1st experimental group due to the lower resistance (Figure 10.2A).

To gradually convert the MFC system to the scale-up IPB system, one and both aeration stones were taken out in sequence. However, after taking out one and both aeration stones at the 3rd and 4th experimental group, the current sharply decreased (Figure 10.2A), even with the added suspended algae to process the photosynthesis for the cathode (OD₆₈₀ = 0.01; initial algal concentration at 1.70 mg L⁻¹). The maximum current generation during daytime of the 2nd channel decreased to the range at 10-14 mA, and then to the range of only 1-4 mA during 3rd and 4th experimental group, respectively (Figure 10.2A). The TC production per day also had the similar

trend, from the TC production at $3592.2 \pm 634.1 \text{ C day}^{-1}$ during the 2nd group, decreasing to $949.4 \pm 150.8 \text{ C day}^{-1}$ during the 3rd group, and finally to the range of $222.4 \pm 152.7 \text{ C day}^{-1}$ during the 4th group (Figure 10.2B). This indicated that the algal photosynthesis could not compensate back for the oxygen supply taken out by the mechanical air pumping for the cathode chamber (Figure 10.2C). For the 2nd channel, the average DO concentration at the 1st and 2nd experiment group was, respectively, at $4.91 \pm 0.29 \text{ mg L}^{-1}$ and $4.84 \pm 0.18 \text{ mg L}^{-1}$, while the DO concentration decreased to $2.58 \pm 0.30 \text{ mg L}^{-1}$ during the 3rd group (Figure 10.2C). The similar DO concentration of the 1st and the 2nd experimental group indicated that the difference of the current generation and high ORR strength in 2nd experimental group had little influence on the DO consumption in cathode. The DO difference between 2nd and 3rd group was mainly due to the difference of oxygen supply between limited algal photosynthesis (low initial algal inoculated concentration at 1.70 mg L^{-1}) and the strong mechanical aeration, and the algal photosynthesis could not compensate back for the removed oxygen supply by one aeration stone bar in this study.

Cold temperature caused the low current generation. The average ambient temperatures from 1st to 3rd groups were $15.3 \pm 2.8 \text{ }^{\circ}\text{C}$, $17.0 \pm 3.8 \text{ }^{\circ}\text{C}$ and $16.0 \pm 4.1 \text{ }^{\circ}\text{C}$, respectively, but the temperature decreased to $7.7 \pm 4.5 \text{ }^{\circ}\text{C}$ and $5.6 \pm 1.8 \text{ }^{\circ}\text{C}$, and even had the minimum temperature at $0.1 \text{ }^{\circ}\text{C}$ in 4th experimental period (Figure 10.2D). The low temperature caused the significant loss of the algal activity on the metabolic process and the algal photosynthesis, and the DO concentration during the 4th group was only at $0.21 \pm 0.59 \text{ mg L}^{-1}$ (Figure 10.2C). It was reported that optimum temperature range for the algal growth of different species was between 20 to 30 $^{\circ}\text{C}$, and if the temperature was below 15 $^{\circ}\text{C}$, the damage or death of the algal cells might occur [450]. The extremely low temperature in the 4th group was detrimental to the algal growth and function. Thus, the current generation and TC production collapsed to the minimum conditions.

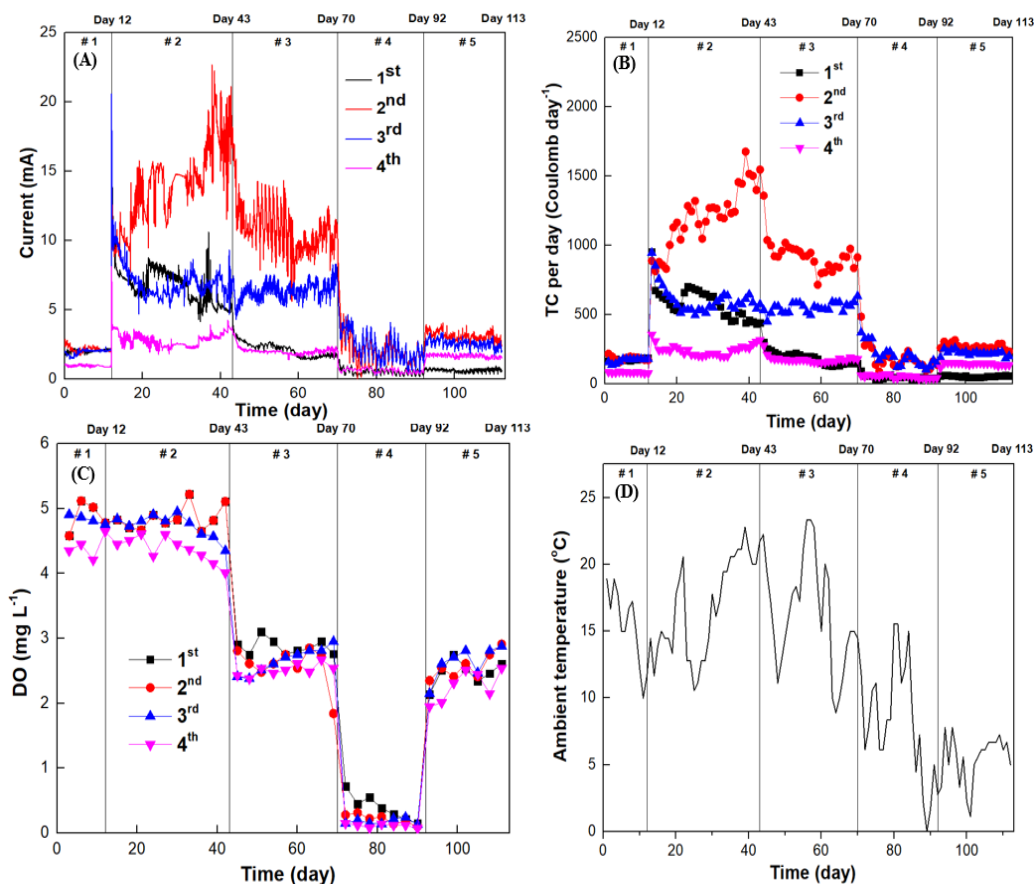


Figure 10.2 Current generation, TC, DO of each channel in the IPB system, and the impact of ambient temperature on the system performance during the entire experimental period: A) current generation (mA); B) TC (Coulombs); C) DO of the catholyte of each channel (mg L⁻¹); D) ambient temperature (°C).

To recover the MFC performance, the aeration with one aeration stone was placed back for each cathode channel, and the DO concentration successfully recovered back to previous level similar as 3rd group, at 2.57 ± 0.20 mg L⁻¹ for the 2nd channel (Figure 10.1C). However, the current generation was hard to recover back to the previous group. Setting the 2nd channel as the example, the current was only in the range of 2-4 mA, much lower to the level in the 3rd group, also using one aeration stone for the oxygen supply. The daily TC production rate for the 5th group was only at 259.1 ± 34.6 C day⁻¹, only 27.3 % of TC production rate of the previous 3rd group, indicating the irreversible damage on the current generation of the MFC. Also, the CE of the entire system during 2nd group was the highest at 6.1 ± 0.5 % among all groups, but decreased to 3.4 ± 0.7 % and 1.0 ± 0.6 % during the 3rd and 4th (Figure 10.3). The CE also could not recover back even placing one aeration stone back in each channel, only at 1.2 ± 0.1 % during the 5th group (Figure

10.3). The low current generation was mainly because of the extremely low temperature, not only causing the inactivity of the algal photosynthesis, but also the unpowerful or impaired electricigens in the MFC anode and the low reaction rate for the ORR and organic degradation [451, 452]. Thus, the low current and TC generation indicated the irreversible breakdown of the scale-up IPB in the cold temperature.

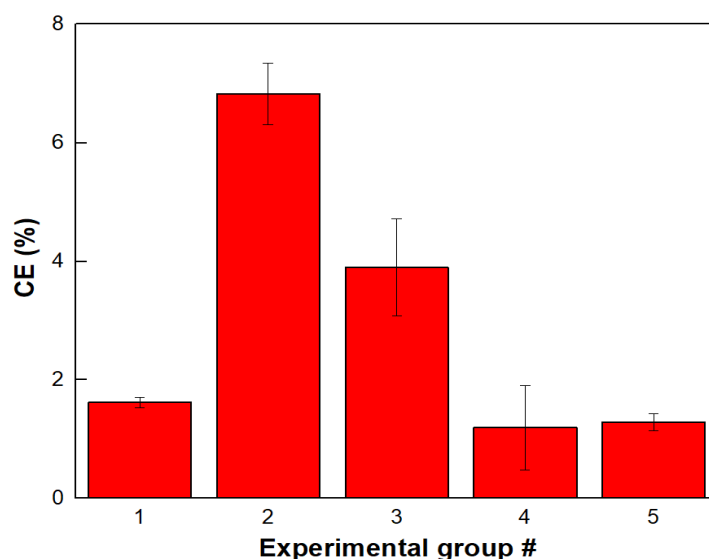


Figure 10.3 CE (%) of the entire IPB system under different experimental groups during this 4-month experiment

The energy generation of each channel and the total generation of entire IPB system were depicted in Figure 10.4A and B, indicating the similar trend, with the highest energy generation during the 2nd experimental group when the sufficient aeration was given but the performance breakdown in the cold temperature during the 4th and 5th group. The total energy generation during the 2nd group reached the maximum among all experimental groups, at $2.47 \times 10^{-3} \pm 0.48 \times 10^{-3} \text{ kWh m}^{-3}$ treated wastewater, while the total energy generations for the 4th and 5th experimental groups were only at $0.12 \times 10^{-3} \pm 0.10 \times 10^{-3} \text{ kWh m}^{-3}$ and $0.16 \times 10^{-3} \pm 0.03 \times 10^{-3} \text{ kWh m}^{-3}$ treated wastewater, respectively (Figure 10.4B). The low energy generation was related to the inability of the algal growth during the 4th and 5th experimental group, based on the dynamic change of the suspended algal concentration in the IPB cathode. From 4th group to 5th group, the average suspended algal concentration greatly decreased. Setting the 2nd group as the example, the algal concentration during the 4th group reached $13.84 \pm 1.70 \text{ mg L}^{-1}$, and finally significantly decreased to $8.55 \pm 2.43 \text{ mg L}^{-1}$ during the 5th group (Figure 10.4C), indicating the malfunction of IPB system on the algal

cultivation. Moreover, compared with other IPB system in the bench scale fed with the real wastewater, the total energy generation of this study was extremely low. Compared with another study directly fed with the real wastewater, with the energy generation at 0.089 kWh m^{-3} with the volume of 2 L [4], the highest total energy generation during the 3rd group in this study, was only about 3% of the previous study.

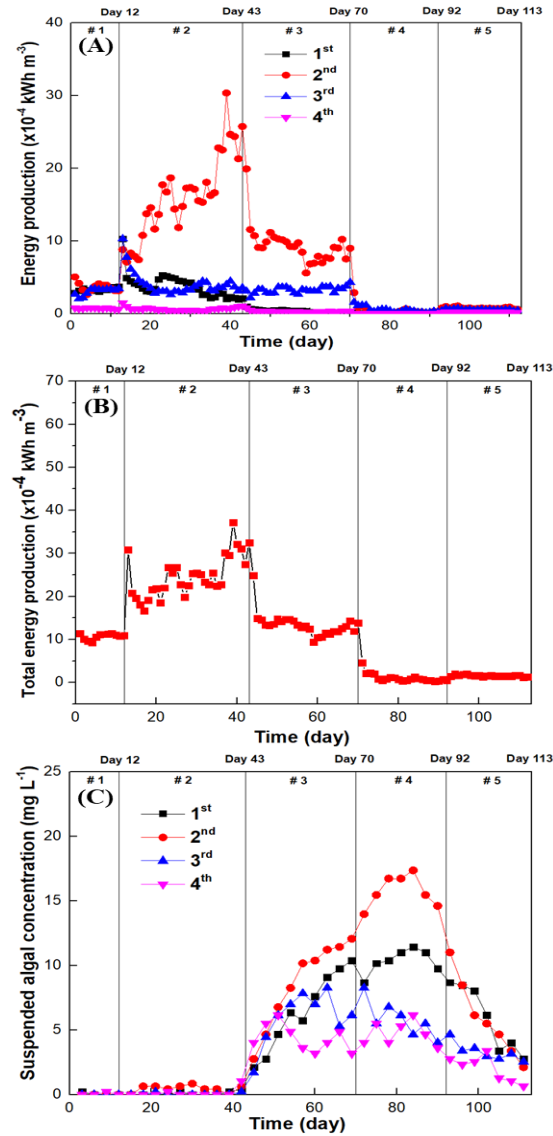


Figure 10.4 Results of electric energy production and the dynamic change of the suspended algal concentration in the IPB system: A) electric energy production from the U-type tubular MFC of each channel ($\times 10^{-4} \text{ kWh m}^{-3}$ treated wastewater); B) total electric energy generation of IPB system ($10^{-4} \text{ kWh m}^{-3}$ treated wastewater); C) the suspended algal concentration (mg L^{-1}) of each channel in the open pond scale-up IPB reactor.

The energy analysis indicated that the outdoor open pond IPB system could not function during winter, and certain heating equipment is needed to maintain the temperature, to improve the system performance and reduce the start-up period. Suitable algal cultivation method was also needed for this study to improve the algal concentration and the photosynthesis strength. The addition of mesh membrane on the catholyte interface might be good option to increase the surface area for the algal attachment and ultimately improve the electric current generation and nutrient removal efficiency [431]. This study indicated that there was still a long way to go to achieve the stable and satisfying scale-up IPB performance towards the actual application.

10.3.2 Organic and nutrient removal

Though the difficulty to generate the electric energy, the effect of the IPB system on the organic and nutrient removal efficiencies was in the good performance. From the influent towards the effluent of anodes and cathodes, the organic and nutrient were generally decreasing. For the COD removal, the COD gradually was decreased along the flow path through the anode of each channel, due the microbial degradation (Figure 10.5A). The $\text{NH}_4^+\text{-N}$ and TN were also continuously decreasing through all the anodes along the flow path. During the flowing condition, most $\text{NH}_4^+\text{-N}$ could migrate through the CEM to the cathode under the electric field and was removed from the anode [374] (Figures 10.5B and C). On the contrast, the TP removal in the anode was minimal, nearly with insignificantly changed concentration for all channels (Figure 10.5D). Overall, the anodes were mainly responsible for the COD removal, basically achieving 70% COD removal for all the anodes (Figure 10.5A).

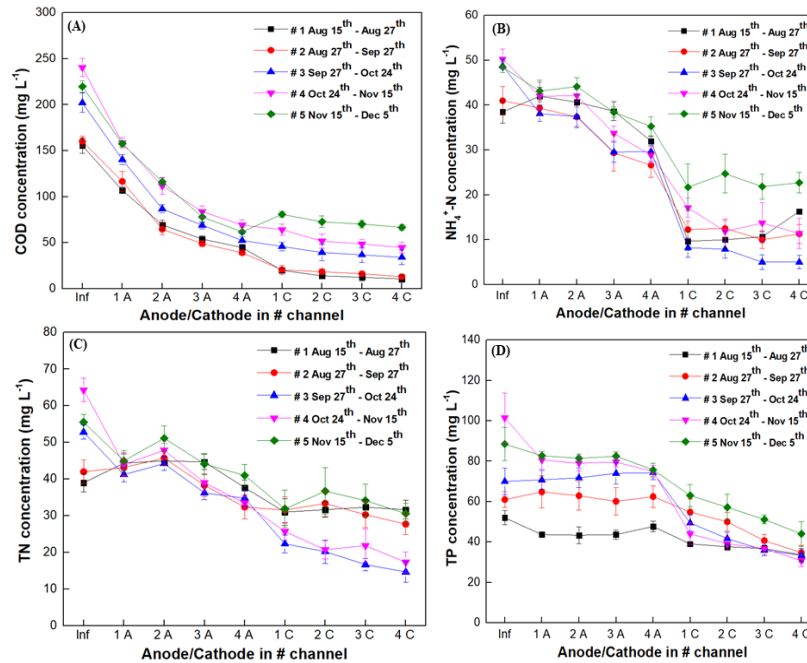


Figure 10.5 The parameters of (A) COD, (B) $\text{NH}_4^+\text{-N}$, (C) TN and (D) TP in the influent, the effluent of anodes and cathodes of each channel under various experimental groups. **Note:** in the x-axis, “Inf” represents IPB influent; the number “1, 2, 3 and 4” represent the channel #, respectively; the letter “A and C” represent the anode and cathode, respectively.

Relatively to the anodic performance, the cathodes were able to not only degrade the organics under the aerated condition, but also remove the nutrients with the algal uptake. For the COD removal, not only the heterotrophic bacteria but also the algae grown in the heterotrophic or mixotrophic mode could help to degrade the organics [57, 453]. However, the outdoor unstable climate might inhibit the performance of heterotrophic bacterial degradation and the algal growth, and thus the COD removal rates in the cathode were inhibited (Figure 10.5A). Also, the algal death might lead to release of the organic, decreasing the COD removal performance [454]. Compared with various experimental groups, the 4th and 5th groups had smaller COD removal rates than the 1st to 3rd groups under the warm temperature (Figure 10.5A), proving the above points. Though the COD degradation rate might be slower in the cathode, the nutrient removal rates in the cathode were satisfying for N and P. For example, the $\text{NH}_4^+\text{-N}$ and TN were removed due to the aerated nitrifiers and algal uptake. The P removal was mainly based on the algal uptake and even the phosphate precipitation along with certain sludge precipitation in the bottom of the algal tank. However, in the long-term operation, the removal rate of the $\text{NH}_4^+\text{-N}$ and TN gradually became lower due to the low temperature for the reaction inhibition (Figures 10.5B and C), and the lower

P removal rate was possible related to the P release due to the algal death and the precipitated sludge [455, 456] (Figure 10.5D). During the last 5th group, the gradually increasing remaining concentration of N and P indicated the inhibition of the cold temperature on the algal growth and reactivity. Moreover, the rainy and cloudy weather led to the insufficient sunlight shed onto the IPB system for all experimental groups, causing the insufficient light absorption for the IPB performance and the unstable removal efficiency [457].

The summary and evaluation of the COD and nutrient removal of the entire system was described and summarized in Figure 10.6. The COD removal efficiency was continuously decreasing from the 1st to 5th groups, from 93.6 ± 2.2 % to 69.6 ± 1.3 % (Figure 10.6). The removal efficiencies of NH_4^+ -N and TN gradually increased from 1st to 3rd groups were increasing due to more mature system cultivation, especially for the 3rd group with the algal addition, with the maximal removal efficiencies of NH_4^+ -N and TN at 89.8 ± 5.4 % and 52.4 ± 4.9 %. However, still due to the cold temperature, the removal efficiencies of NH_4^+ -N and TN decreased to 53.2 ± 4.7 % and 44.9 ± 5.0 % (Fig. 6). Similarly, for the TP removal, the removal efficiency decreased from 69.7 ± 2.9 % at the 3rd group to 50.3 ± 6.9 % at the 5th group (Figure 10.6). Thus, the performance of the IPB system on the wastewater treatment gradually deteriorate along with the decreasing temperature. The reason for the deteriorating performance of the scale-up IPB system was due to the change of the external climate, leading to the inactive performance of electricigens and algae, and the insufficient light emission during the clement weather. Also, for the low P removal in the later period, the precipitated sludge (possibly co-precipitated with Ca-P) due to algal photosynthesis might accumulate in the initial period, and then released the P into the algal tank to inhibit the P removal efficiency [456]. Facing various difficulties on the nutrient removal, to summarize, there is still a long way to go to operate the scale-up IPB system.

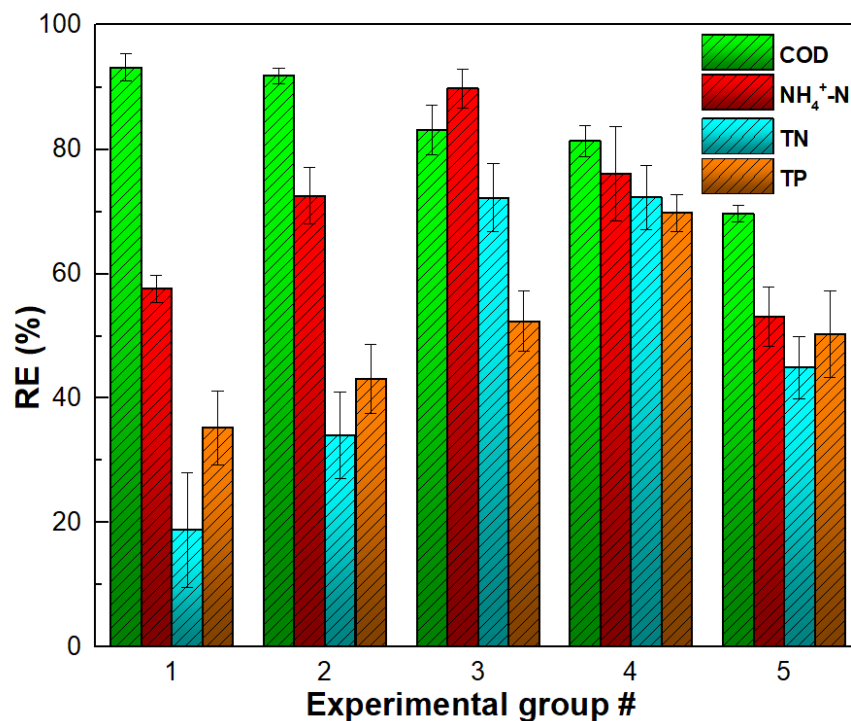


Figure 10.6 Total removal efficiency (%) of COD, NH₄⁺-N, TN and TP of entire open pond scale-up IPB system.

10.3.3 Perspectives

The long-term operational IPB systems was proved with many problems needing to be solve, and multiple solution are needed to be proposed to try to find a suitable solution for these problems. First, the algal tank (cathode) might need equipment for the on-time scrubbing or refreshing for the microbial consortia and remaining sludge, to guarantee the performance of the IPB system on the nutrient removal. To improve the system performance, the addition of the algal scrubber could be added to combine the system, to harvest the suspended algae and tend to remove the accumulating sludge (with accumulated P) in the bottom of the cathode tank during certain time interval to reduce the internal nutrient release and the inhibition on the nutrient removal efficiency [456]. Second, another solution is to place the heater in the cathode, or adopt the waste heat from other reactors, to guarantee the warm temperature of the catholyte (at least > 15 °C), to improve the algal growth and boost the IPB performance. Third, if there is no mandatory need to operate the system for one entire year, the IPB system can be operated only under the environment with favorite temperature, to guarantee the IPB system to function well during that period and reduce the daily operational cost [457]. Fourth, the heat flux is related to the reactor geometry and location.

For the reactor geometry, it was reported that the small channel width and thicker panel to separate the channel could reduce the heat loss during the performance of algal tank, to finally reduce the number of the cold days for the system performance [457]. Thus, the reduction of the channel width might increase the system resistance against the cold and windy weather. Fifth, avoidance of placing the system in the location with high latitude to avoid cold temperature also needs consideration to guarantee the IPB performance. The town in Appalachian, Blacksburg in Virginia, was chosen to place the IPB system and operate the experiment in this study, and the cold temperature ($< 15\text{ }^{\circ}\text{C}$) frequently occurred during November to March, leading to huge problems on the normal operation, and thus more tropical and suitable places might be better to operate the system, to receive stronger light illumination and improve the heat flux into the reactor. Sixth, specific strains with high resistance of the cold temperature can be used to inoculate into the IPB system, to increase the system compatibility in the cold temperature [452, 457]. Last, it was reported that the vertical scale-up reactor was more efficient to produce more algae in the algal pond [48]. Vertical photobioreactors possessed higher areal productivities than the conventional horizontal photobioreactors because of the higher photon flux densities on the reactor surface of the vertical reactor [48]. The flat panel photobioreactor set as the vertical orientation was reported with the optimal photosynthetic efficiency and volumetric productivities due to the short optical path [48], and the reactor design of the IPB system in the future can focus on the shape of vertical flat panel photobioreactor to construct for higher illumination receiving efficiency.

10.4 Conclusion

The scale-up IPB system was constructed in the shape of open-pond system, on purpose of treating the wastewater discharged from the university. The evaluation of the electric energy generation and wastewater treatment performance was set as the standard to investigate the scale-up IPB performance. However, due to the low initial algal concentration and slow growth rate due to the inclement weather, the IPB performance with the algal photosynthesis could not have the comparable performance in the electricity generation with the conventional MFCs with the mechanical aeration. Furthermore, the cold temperature made irreversible breakdown for the IPB performance, and the electric generation and wastewater treatment efficiency were inhibited, and could not function during winter. This study had a trial to test the performance of the scale-up IPB

system, and several problems and bottlenecks were discovered, to give sufficient information and references to further improve the system performance in the future.

Chapter 11 Perspectives

This dissertation collectively summarizes all the information about my PhD studies, and these studies are focused on the fundamental mechanisms, performance evaluation, optimization process and application development about BES, algal reactor, and the integration of both systems as IPB system. The application results and promising perspectives indicate that the BES has great promise for the future to be developed and improved. This chapter is to demonstrate that what functions or aspects should be focused to develop to achieve better sustainable energy recovery by the strong system.

11.1 Cathode material improvement

In Chapter 2, the Ni-carbon coated fiber was introduced to increase the cost-effectiveness on the energy generation. The cathode modification should also consider about how to increase the algal performance in the cathode, such as improving the surface area for the algal attachment on the cathode material, to finally improve the algal growth rate and energy recovery. In Chapter 9, the introduction of the mesh membrane achieved the purpose, but the mesh membrane still has the certain distance from the cathode material, and thus the oxygen transfer from the algal photosynthesis still faces certain barrier due to the transferring difficulty, especially for the scale-up reactor. The cathode material could combine with more carbonaceous modification, such as introduction of carbon nanotube, carbon nanofiber to improve the durability of the cathode material against the wastewater corrosion and also increase the surface area for the algal attachment [458, 459], to increase the oxygen transfer efficiency. Also, the stronger ORR reaction in the cathode can reduce the system resistance to improve the electricity generation. Thus, the electric energy generation can be improved by using the cost-effective catalyst on the carbonaceous material.

11.2 Mathematical modeling development

In Chapter 8, a mathematical model was introduced to make the simulation for the IPB system, and gave the optimization strategy. Modeling is the good strategy to give the system prediction to help to find out the problems and methods to improve the system performance. However, for the future application value, the mathematical model can be developed to give more information about the effect of other operational factors on the system performance, such as illumination strength

and the possible inhibition effect from the excessive illumination. The suitable illumination intensity and period can improve the growth of algal biomass [460], and affects the composition of the algal community [461, 462]. while excessive illumination will conversely inhibit algal growth due to photoinhibition [463, 464]. Also, oxygen saturation is reported to inhibit photosynthetic activity [465], and obtaining more knowledge about how to adjust oxygen concentration is beneficial for both current generation and algal growth in the IPB system. Finally, the IPB model in Chapter 8 did not cover the impact of algal-bacterial assemblage on IPB performance. Various algae inoculation will change the community composition and performance. Integration of the factors of algal-bacterial assemblage into model can give directly numerical indicator on what the main working mechanism of the microbial parts (e.g., internal nutrient flux, synergistic relationships to benefit both growth). The following table is summarized to indicate what model equations can be added to the original model to cover the effect of the illumination effect, oxygen loss and the effect of the bacterial growth.

Table 11.1 New equations added into original IPB model

Function	Equation	References
Illumination effect on the constant of maximum growth rate of algae	$\mu_{max-AB} = \mu_{alg} \left(\frac{I}{K_I + I} \right)$ μ_{alg}: fundamental maximum algal biomass growth rate not modified by illumination; K_I: half-saturation constant for illumination	[409, 410, 466-468]
Transfer of oxygen from liquid to atmosphere	$\frac{dDO}{dt} = A + K_L a_0 (S_0^{sat} - DO)$ $S_0^{sat} = MW_{O_2} H_{O_2}(T) P_{pO_2}$ A: the original mathematical expression fraction for DO in preliminary study; $K_L a_0$: surface mass transfer coefficient of oxygen between liquid and atmosphere; S_0^{sat}: saturation concentration of oxygen in water; MW_{O_2}: molecular weight of oxygen (32 g/mol); $H_{O_2}(T)$: Henry's law constant at temperature T; P_{pO_2}: oxygen partial pressure.	[469, 470]
Dynamics of bacterial growth	$\frac{dB}{dt} = \mu_{max-B} \left(\frac{S_c}{S_c + K_{C-B}} \right) \left(\frac{C_{NH_4-N-c}}{C_{NH_4-N-c} + K_{NH_4}} \right) \left(\frac{DO}{DO + K_{DO}} \right) B - \frac{F}{V_c} B$ B: bacterial concentration in cathode; μ_{max-B}: maximum growth rate of bacteria; K_{C-B}, K_{NH_4}, K_{DO}: half-saturation constants for bacterial consumption on organic, NH4-N and oxygen	[469]

The model modification is possible to easily, directly and clearly exhibit the impact of various algae inoculations, and show the algal-bacterial interaction on IPB performance (e.g., mass flux, current generation, growth condition), which is a good starting point for further research in describing the dynamic behavior of the consortia of algae-bacteria.

11.3 Scale-up process improvement

In chapter 10, the trial to construct the scale-up IPB reactor to treat the real wastewater was introduced. However, the results were not good on the electric generation due to the cold temperature, and the activity of the algae was suppressed. Thus, alternative species which has strong tolerance ability against the cold temperature are better to be introduced to help the IPB system to maintain the performance under the coldness. It was reported that diatoms were more dominant under the low light condition and cold temperature (7-11 °C) [471]. The strong resistance against the coldness potentially enables the diatom introduction into the cathode to recover the energy production under the cold condition. Furthermore, the high oxygen production rate of the diatoms due to their strong irradiance adsorption ability can potentially facilitate the oxygen production and transferring to promote ORR in the IPB system [472].

Introduction of the mesh membrane on the top of the tubular MFC anodes in the scale-up IPB system (similar as Figure 10.1), may help to increase the algal growth rate by giving more surface area, and reduce the illumination loss by placing the mesh membrane on the water interface [422]. Further, combination of the mesh membrane inside the IPB reactor can give more media surface for the algal attachment and facilitate the algal harvesting without disturbing the placement of the tubular MFC anodes. Thus, the system stability can be improved, and the algal harvesting efficiency can be improved due to the high growth rate of the algal biofilm [188].

11.4 Techno-economic analysis by modeling to calculate cost-effectiveness

Though the scale-up process of IPB system was constructed and tested to evaluate the system performance and give information about if the IPB system could actually work for various types of wastewater. Also, the cost-effectiveness should be assessed to investigate the actual application value of the IPB system, to give information about the benefits or profits the system can give. However, the evaluation process is complicated and time-consuming, due to the high laboring requirement for the frequent testing and the difficulty to compare with other system performance due to the lack of the uniform standard.

For the standard unit, the energy recovery is a suitable option to represent the relationship between the actual inputs and the outputs of the system. A new concept, net energy recovery (NER), is introduced to represent the difference between energy consumption and production [29], to indicate the actual benefit that the system can bring out. The NER comparison can directly give the information about which system or operational mode is better to be adopted to save money or not. Thus, in the future, the energy data must be needed to report the IPB system, for the convenience to compare their cost-effectiveness.

Next step is to improve the NER, and finding a suitable strategy is important to achieve the system promotion. To give better directions for the system improvement, the development of the modeling strategy is still good option to complete the purpose. Previously, uniformly economical comparison standard is lacked for the cost-effectiveness of IPB (e.g., total profit output/total cost input). A uniform techno-economical model to compare capital/operation cost and bioenergy revenue of IPB system is a potentially strong tool to facilitate engineers to know the strategies to enlarge the profit recovery from IPB in long-term operation (e.g., five years). Knowledge of the respective construction and operational cost of individual components (algal bioreactor and MFC) is foundation for techno-economic model construction. Multiple studies have been done on capital cost of MFC and algal bioreactor, respectively. For example, in MFCs, it was reported that total cost for a 200 L tubular MFC was about \$58 to treat actual domestic wastewater per gallon per day, and the total power output was 0.039-0.081 W [191]. For the algal bioreactor, configurations significantly impact irradiance absorption efficiency and algal growth rate. Closed systems (e.g., flat-panel or tubular photobioreactor) have higher biomass productivity ($40\text{-}195\text{ g m}^{-2}\text{ day}^{-1}$) than open pond system ($20\text{-}30\text{ g m}^{-2}\text{ day}^{-1}$) due to easier condition control [411], but causes a large amount of capital and operational cost [51, 473, 474]. The balance between cost and profit of IPB system is needed, and it should be solved by constructing the techno-economic model to give information on the energy and cost balance.

There are some potential options to consider about the construction of the techno-economic model. One uniform standard concept can be used to compare cost analysis of various IPB systems: net present value (NPV) [475, 476]. The purpose of the NPV is to compare the profit feedback after starting system operation for a certain time and the initial capital cost. The calculation method for

NPV is to sum all the future cash flows (CF) to determine the present value, using the following equations:

$$NPV = -CF_0 + \frac{CF_1}{(1+r)^1} + \frac{CF_2}{(1+r)^2} + \dots + \frac{CF_n}{(1+r)^n} = -CF_0 + \sum_{t=1}^n \frac{CF_t}{(1+r)^t}$$

The IPB system is defined as worthwhile by the NPV rule if its $NPV > 0$. In this equation, CF_0 represents initial capital cost, while CF_t represents profit (derived from previous model) at the year/month t , r is the yearly/monthly discount rate (set from 0.01 to 0.10) to represent the system degradation constant, and n represents the life (year/month) of the IPB system. The maximum value of yearly discount rate is set at 10% to predict the worst profit recovery condition as system baseline [477]. NPV can directly show the probability to obtain profit, to give engineers a comprehensive picture to optimize the IPB performance based on various configurations and operational conditions.

To extend the application of the new model for cost analysis of IPBs at various sizes or configurations, the empirical techno-economic equation intended for size transformation is valuable, shown as below [478, 479]:

$$New\ Cost = (Base\ Cost) \left(\frac{New\ Size}{Base\ Size} \right)^{0.6}$$

The equation can estimate the cost of IPBs at new size from the base size (total volume at 1500 mL in this study). However, the model is limited for bench-scale IPB system (at risk to estimate for size >10L), and the configuration should be the same when estimating the IPB cost at other sizes. The techno-economic model can give uniform standard to directly compare the cost effectiveness of the IPBs under various configurations, giving a comprehensive picture to help the engineers design and construct the IPBs.

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Appendix

Appendix A for Chapter 1

Table A.1 General stereotype ODE.

Main Equations	Applications	Assumption (All in Reference)	Founders
$\frac{dX_B}{dt} = \frac{\phi}{v_B} (X_{in} - X_B) + r_{X,B} + r_{det} \frac{A_F}{v_B} - r_{ata} \frac{A_F}{v_B}$ $\frac{dS_B}{dt} = \frac{\phi}{v_B} (S_{in} - S_B) + r_{S,B} + \frac{1}{v_B} \int_{v_F} r_{S,F} dV + \frac{1}{v_B} \int_{A_F} r_{S,E} dA$ (Assumption: F,G) X_B: biomass concentration; X_{in}: initial biomass concentration in bulk liquid; ϕ: influent liquid flow rate (zero in batch mode); v_B: bulk liquid volume; $r_{X,B}$: net biomass growth rate in the bulk; A_F: anode surface; r_{det}: biofilm detachment rate; r_{ata}: biofilm attachment rate; S_B: solute concentration; $r_{S,B}$: net substrate reaction rate in the bulk; $r_{S,F}$: net substrate reaction rate in the biofilm; $r_{S,E}$: electrochemical rates of solute component change on the electrode surface	Predict concentration of microorganisms and soluble components in bulk liquid based on mass balance	A,C,D,E,F,G	Picioreanu et al [135]
$\frac{dS}{dt} = D(S_0 - S) - q_a X_a - q_m X_m$ $\frac{dX_a}{dt} = \mu_a X_a - K_{d,a} X_a - \alpha_a D X_a$ $\frac{dX_m}{dt} = \mu_m X_m - K_{d,m} X_m - \alpha_m D X_m$ (Assumption: F,G) S: substrate concentration; S_0: influent substrate concentration; D: dilution rate ($D = \frac{\text{Flow rate}}{\text{Volume}}$);	Predict the concentration of targeted substrate, anodophilic and methanogenic microorganisms, and embody the competition between	A,C,D,F,G,I	Pinto et al. [150]

X_a : concentration of anodophilic microorganism; X_m : concentration of methanogenic microorganism; q_a, q_m : substrate consumption rates of anodophilic and methanogenic microorganism; μ_a, μ_m : growth rates of anodophilic and methanogenic microorganism; $K_{d,a}, K_{d,m}$: decay rate of anodophilic and methanogenic microorganism; α_a, α_m : dimensionless biofilm retention constants of anodophilic and methanogenic microorganism	these two kinds of microorganisms		
$\frac{dM_{ox}}{dt} = -Yq_a + \gamma \frac{I_{MFC}}{mF} \frac{1}{VX_a} \text{ (Assumption: C)}$ $\mu_a = \mu_{max,a} \frac{S}{K_{S,a}+S} \frac{M_{OX}}{K_M+M_{OX}} \text{ (Assumption: H)}$ M_{ox}: oxidized mediator fraction per anodophilic microorganism; Y: mediator yield; q_a: substrate consumption rate of anodophilic organism; γ: mediator molar mass; I_{MFC}: MFC current; m: number of electrons transferred per mol of mediator; F: Faraday constant; V: bulk liquid volume; X_a: concentration of anodophilic microorganism; $\mu_a, \mu_{max,a}$: growth rate and maximum growth rate of anodophilic microorganism; S: substrate concentration; $K_{S,a}, K_M$: half-saturation (Monod) constant	Predict the impact of mediator to carry electrons from oxidizer to reducer to achieve the current generation.	A,C,D,F,G,H,I	Pinto et al.[150, 165]

Note: capital letter in assumption column refers to Table A3.

Table A.2 Applications of ODE in MFC modeling.

Stereotype ODE Source	Equation Addition or modification on Stereotype (Only show important equations and assumptions)	Application	Assumpti on (All in Reference)	Reference

Pinto et al.	$\frac{dC_{salt,m}}{dt} = D_{salt}(C_{salt,in} - C_{salt,m}) - d(C_{salt,m} - C_{salt,a}) - d(C_{salt,m} - C_{salt,c}) - \frac{I_{MDC}}{F * V_{salt}}$ $\frac{dC_{salt,a}}{dt} = d(C_{salt,m} - C_{salt,a}) - DC_{salt,a}$ $\frac{dC_{salt,c}}{dt} = d(C_{salt,m} - C_{salt,c})$ (Assumption: F,L) $C_{salt,m}, C_{salt,a}, C_{salt,c}$: salt concentrations in the salt, anode, and cathode compartments; D_{salt}: dilution rate in salt chamber ($D_{salt} = \frac{Q_{flow,salt,m}}{V_{salt}}$); d: membrane salt transfer coefficient ($d = \frac{f * A}{b * V_a}$, f: diffusion coefficient; A: membrane surface area; b: membrane thickness; V_a: anode volume); I_{MDC}: current through the circuit of MDCs; V_{salt}: volume of salt chamber;	Estimate the salt concentration under various conditions to optimize MDC performance	A,C,D,F, G,H,L	[166, 167]
Pinto et al.	$\frac{dC_{salt,a}}{dt} = \frac{Q_{anode,in}}{V_{anode}} C_{salt,a,in} - \frac{Q_{anode,out}}{V_{anode}} C_{salt,a} - \frac{J_s}{V_{anode}} A_m$	Estimate the impact of water flux through forward osmosis (FO) on the performance of entire system		[374]

	$\frac{dC_{salt,c}}{dt} = \frac{Q_{anode,in}}{V_{cathode}} C_{salt,c,in} - \frac{Q_{cathode,out}}{V_{cathode}} C_{salt,c} + \frac{J_s}{V_{anode}} A_m$ (Assumption: F) $C_{salt,a}, C_{salt,c}$: salt concentration in the anode and cathode; $C_{salt,a,in}, C_{salt,c,in}$: salt concentration in the anode and cathode influent; $Q_{anode,in}, Q_{anode,out}$: influent and effluent flow rate; $V_{anode}, V_{cathode}$: volume of anode and cathode; J_s: reverse salt flux; A_m: membrane area		A,C,D, F,G,H,J	
Pinto et al.	$\frac{dm}{dt} = Q_{out}X - J_{air}m\beta \frac{m}{K_{air} + m}$ $\frac{d\beta}{dt} = \gamma\beta$ (Assumption: F) m: cake layer mass; Q_{out}: effluent rate; X: concentration of suspended solids; J_{air}: air cross-flow; β: resistance of the cake to detachment; K_{air}: half-saturation coefficient of air flow; γ: coefficient for cake detachment	Predict the the dynamic change of biomass “cake” on the microbial membrane reactor.	C,D,F,H	[480]
Picioreanu et al.	$A_{ano}Z \frac{dC_{i-glu}}{dt} = A_{ano} \frac{D_{glu}}{Z} (C_{i-1-glu} - C_{i-glu})$	Finite difference		

$A_{ano}Z \frac{dC_{i-H^+}}{dt} = A_{ano} \frac{D_{H^+}}{Z} (C_{i+1-H^+} - C_{i-H^+}) - A_{ano} \frac{D_{H^+}}{Z} (C_{i-H^+} - C_{i-1-H^+}) + 2R_{Ai-glu}A_{ano} + 7R_{Ai-ace}A_{ano}Z + 13R_{Ai-pro}A_{ano}Z + 2 * 0.29R_{Ai-H_2}A_{ano}Z$ (Assumption: B,K) $C_{i-glu}, C_{i-1-glu}$: glucose concentration in i_{th} and $(i-1)_{th}$ layer, A_{ano}: surface area of anode electrode; Z: biofilm thickness; $R_{Ai-glu}, R_{Ai-ace}, R_{Ai-pro}, R_{Ai-H_2}$: local consumption rates of glucose, acetate, propionate and hydrogen in each finite layer; D_{glu}, D_{H^+}: Diffusion coefficient of glucose and proton	approach is applied to divide biofilm into multiple finite layers to predict chemical kinetics of glucose and proton between biofilm and bulk solution	B,D,I,K	[140]

Note: capital letter in assumption column refers to Table A3.

Table A.3 Assumption Lists for ODE modeling.

Assumption Letter	Assumptions	Limitations to be Solved by Assumption
A	Simple substrate (i.e., acetate) is chosen to be electron donor and carbon source due to complete degradation to CO ₂ .	Uncertainty exists for the reaction rate for step-by-step degradation of complex substrate[481]. Acetate takes only one step of degradation to produce CO ₂ to make model available.
B	Glucose degradation is assumed to have two main designed steps with fixed chemical reactions to only produce hydrogen, acetate and propionate.	Uncertainty exists for products from the degradation of glucose[482]. Two-degradation eliminates the uncertainty

C	Certain concentration of redox mediator exists from the beginning in the bulk liquid, and redox mediator produced by anodophilic microorganism completes electron transfers in the model.	Different electron transferring mechanisms may exist for anodophilic bacteria[288]. Assumption of only one electron transferring mechanism to work can eliminate the interference of other mechanisms in the model
D	Constant value of cathode potential is set due to model focus on anode chamber.	The model focuses on the problem of anode. Eliminating the limitation of cathode can reduce the external interference on anode performance.
E	Ammonium is chosen to be nitrogen source.	Ammonia is common nutrient for the bacteria to utilize [483-485]. To eliminate the interference of other nitrogen type, ammonia is assumed to be nitrogen source.
F	Substrate and microorganism in the bulk solution and electrode biofilm are assumed to be ideally mixed or uniform.	Distance to the electrode and substrate distribution has effect on system performance. Assumption can reduce the interference and overcome the model limitation.
G	Acetate can feed two competing microbial populations independently, anodophilic and methanogenic bacteria. Growth rate and methane production of methanogenic bacteria depends on acetate concentration.	Multiple types of bacteria and substrate complicates the reaction kinetics, and model cannot simulate the substrate degradation and growth of all bacteria. Classification of only two types of bacteria can overcome the model limitation.
H	Multiplicative Monod kinetics is used to describe growth kinetics of anodophilic microorganisms, whose growth rate depends on acetate and intracellular mediator.	Multiplicative Monod kinetics can simplify and control the limiting factors for the impact on growth rate of bacteria, to make model workable.
		pH and temperature can influence the system environment and reaction

I	Temperature or pH is considered fully controlled and kept constant (not the limiting factor in the system).	kinetics, to finally influence the substrate degradation. Keeping constant of temperature and pH can eliminate the influence of these two factors to make model workable.
J	Anodophilic microorganism is the dominant community.	Multiple bacteria exist in the system, and setting anodophilic microorganisms as dominant community is to use the model to simulate the impact of anodophilic microorganisms in electricity generation, since other bacteria are unimportant and relatively rare.
K	Consumption rate of substrate in the bulk liquid is assumed to be negligible relatively to consumption rate within the electrode biofilm.	To simulate the degradation rate of substrate throughout the biofilm, the substrate degradation in the bulk solution should be neglected to reduce the interference of bulk solution on the system.
L	Electromigration of every electron between two electrodes pulls out a salt ion from salt chamber.	Since the salt is sodium chloride (NaCl) in the experiment, the assumption is reasonable because Na^+ and Cl^- are monovalent ion. The assumption that electromigration of every electron pulls a salt ion facilitates the mathematical calculation for the problem.

Appendix B for Chapter 2

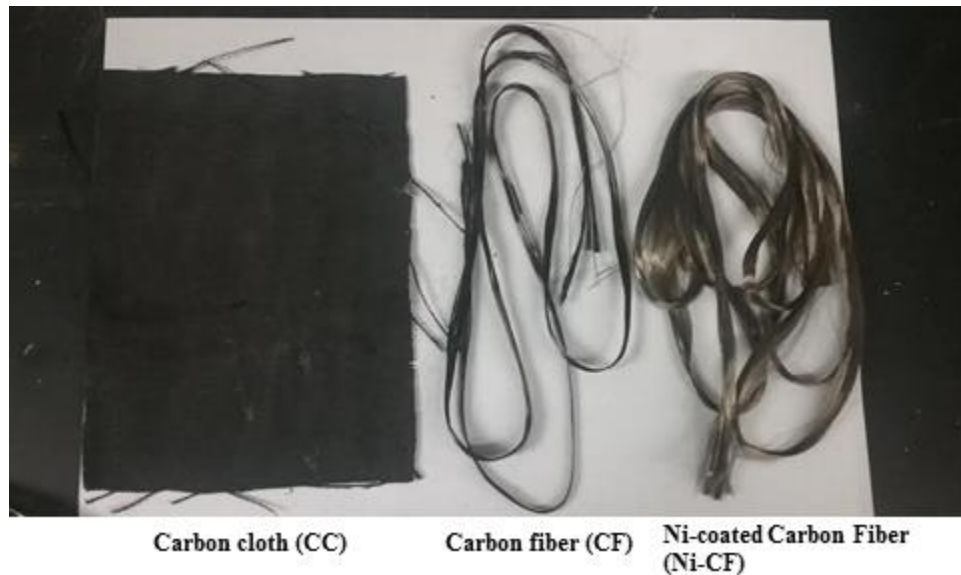


Figure B.1 Pictures for the supporting materials for cathode electrode in this study.

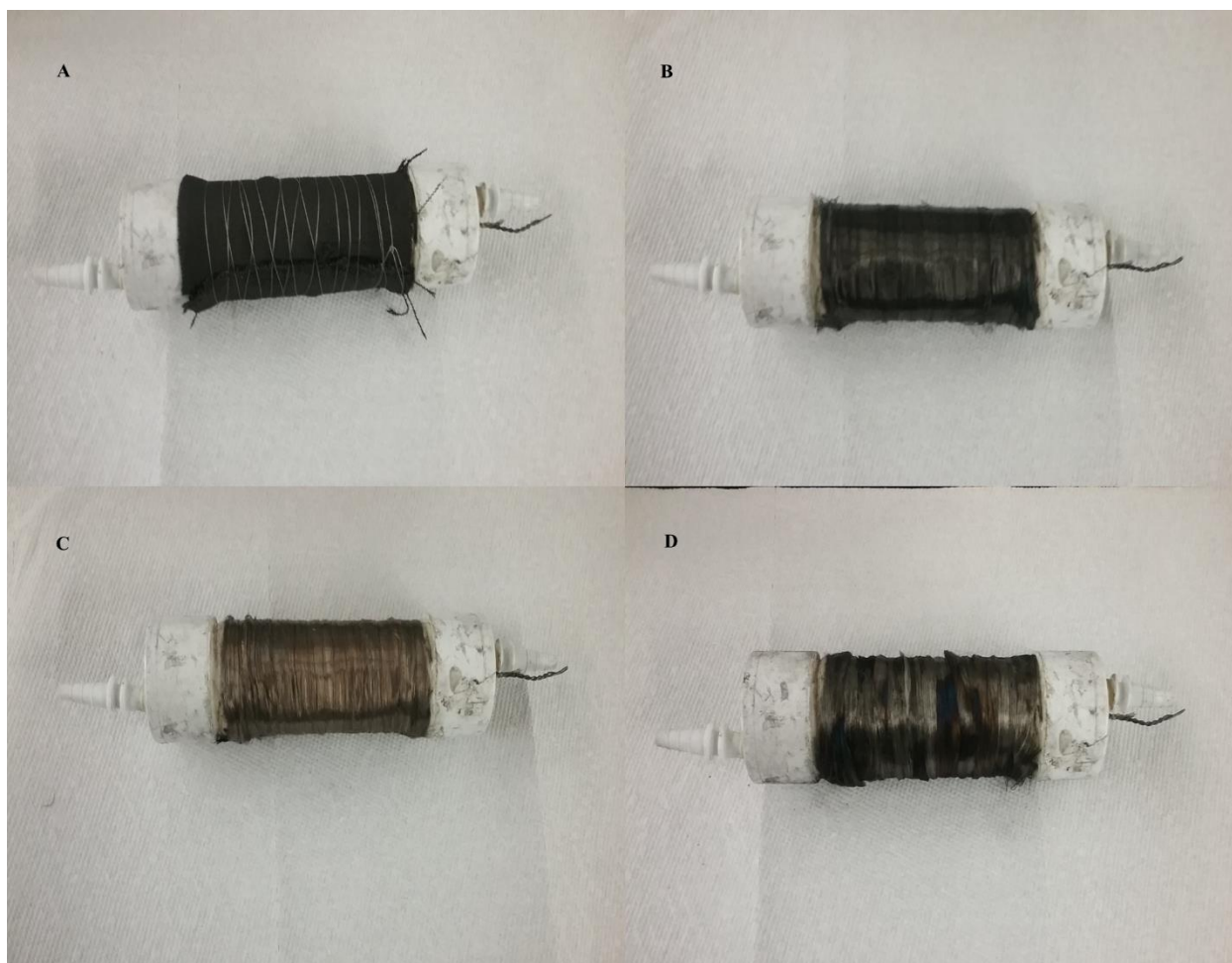


Figure B.2 Pictures of individual tubular MFCs wrapped with various cathode material:(A) AC/CC; (B) CF; (C) Ni-CF; (D) AC/Ni-CF. Note: AC/CC represents activated carbon coated on carbon cloth; CF represents carbon fiber; Ni-CF represents Ni-coated carbon fiber; and AC/Ni-CF represents activated carbon coated on Ni-coated carbon fiber.

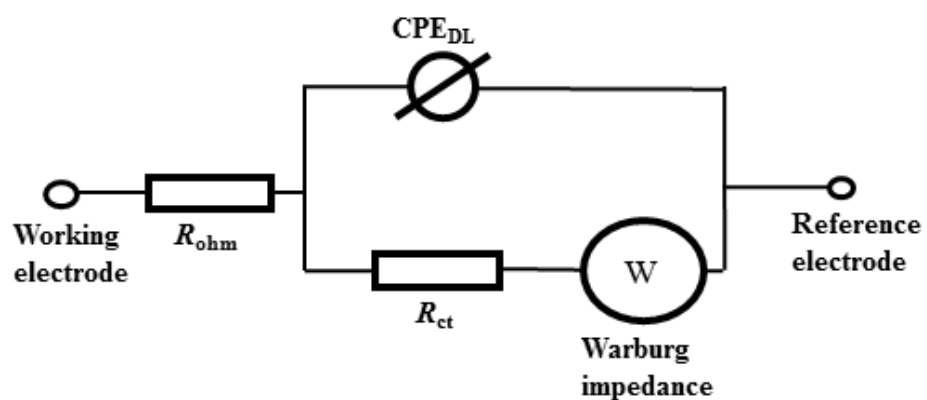


Figure B.3 Equivalent circuits used for the analysis of impedance data in this study. Note: R_{ohm} represents ohmic resistance; R_{ct} represents charge transfer resistance; W represents Warburg impedance; CPE_{DL} represents the constant phase element for double layer.

Appendix C for Chapter 3

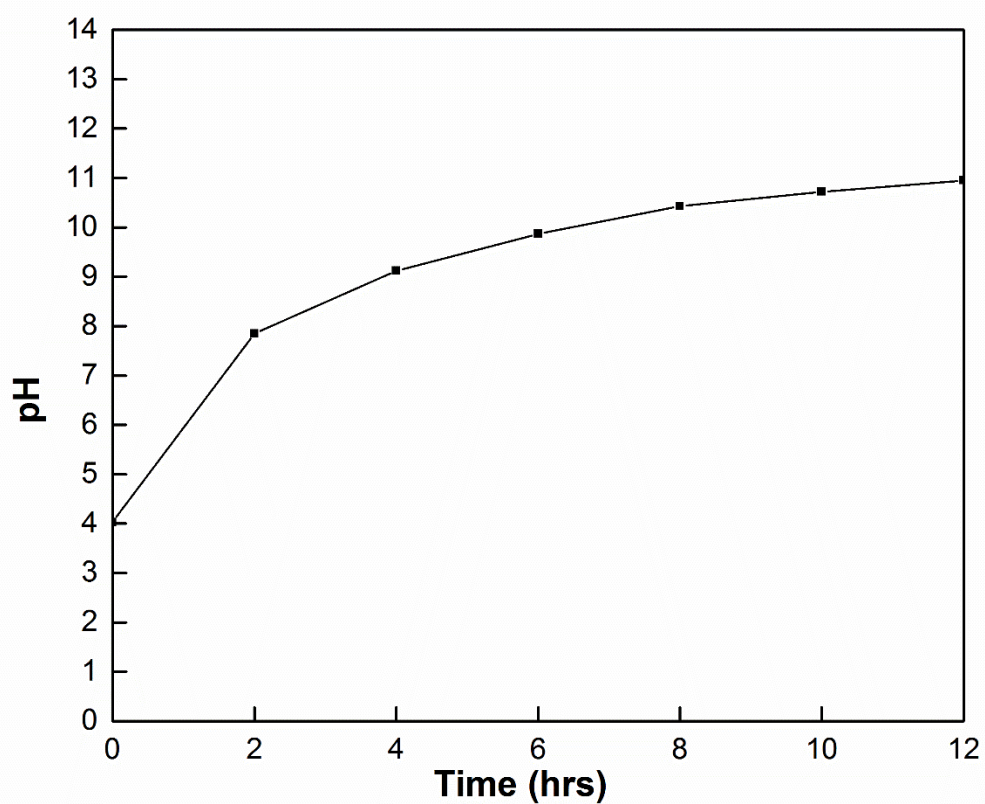


Figure C.1 pH change of catholyte in the scale-up MEC reactor in a 12-hr cycle under the operation condition (flow rate: 12.5 mL min^{-1} ; recirculation rate: 0 mL min^{-1} ; external resistance: 1 ohm).

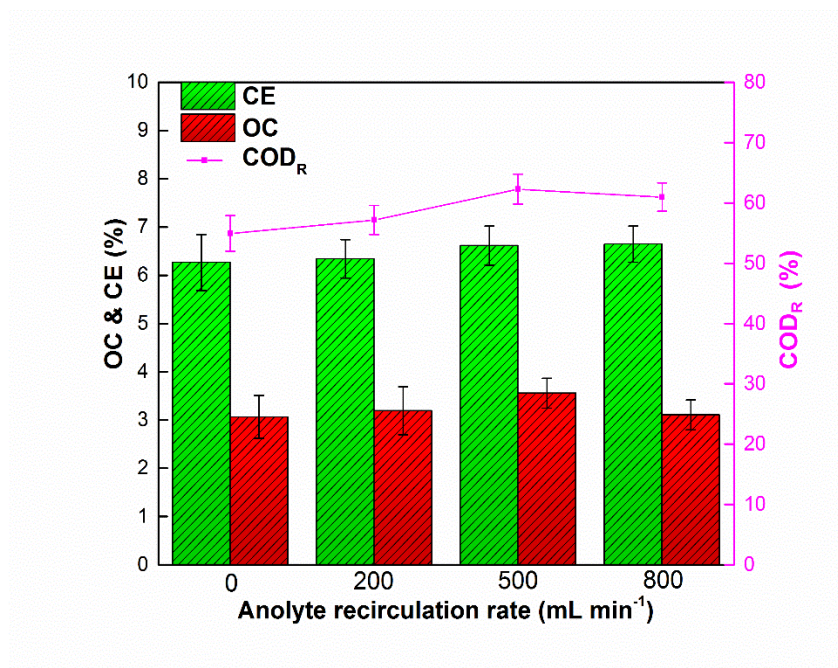


Figure C.2 CE, OC and COD removal efficiency of the scale-up MEC under various anolyte recirculation rates (i.e., 0, 200, 500, 800 mL min⁻¹)

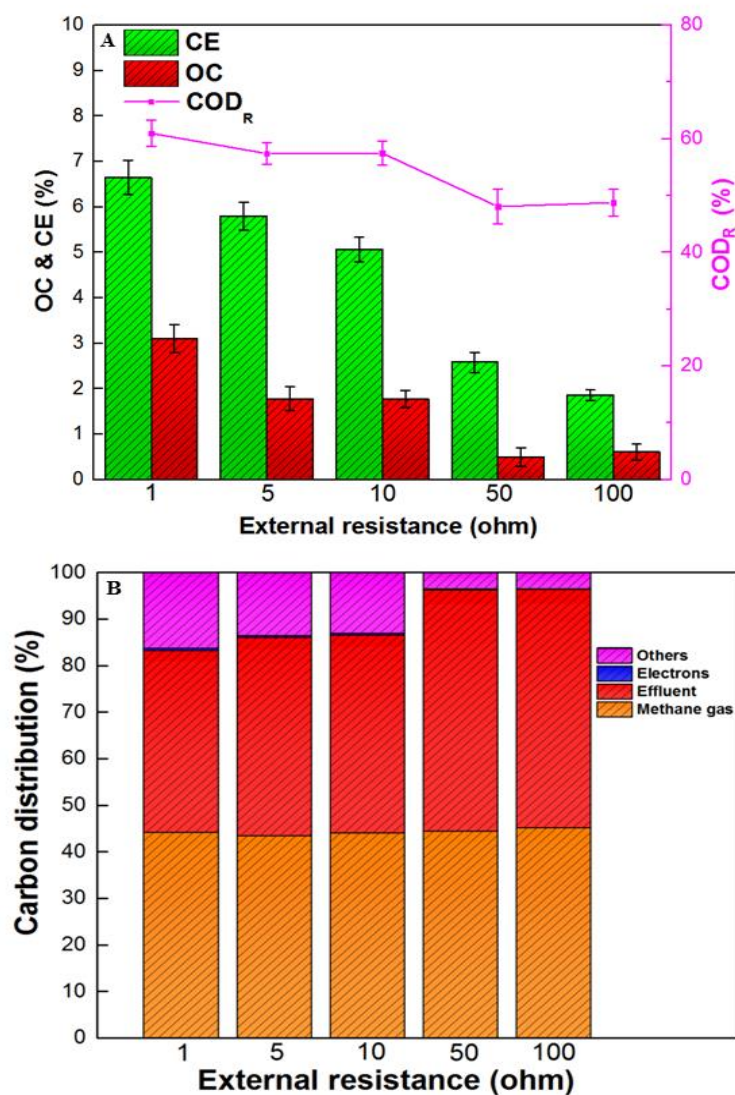


Figure C.3 Performance of the scale-up MEC under various external resistances (1, 5, 10, 50, 100 ohm) under HRT and recirculation rate at 24 h and 800 mL min⁻¹ respectively: (A) CE, OC and COD_R under various external resistance; (B) carbon distribution in different components.

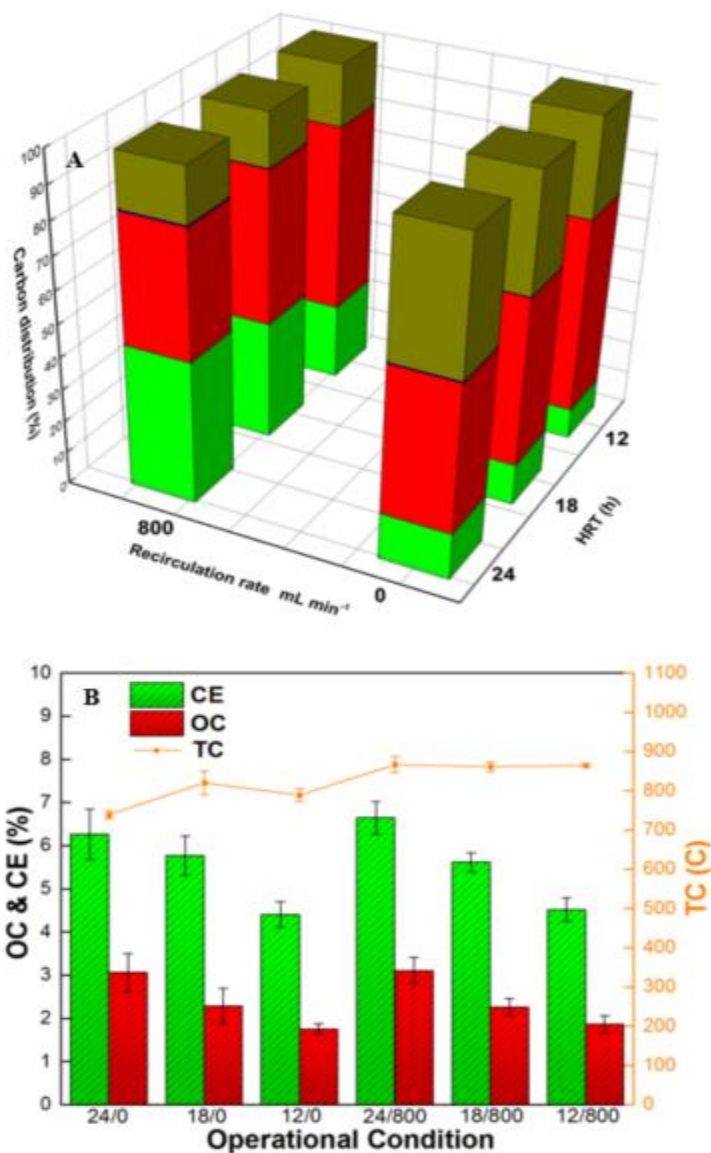


Figure C.4 Performance of the scale-up MEC under orthogonal experimental design of various HRTs (24, 18 and 12 h) and recirculation rate (0 and 800 mL min⁻¹): (A) carbon distribution shown in stacked bars (green, red and brown bars represent carbon in gaseous CH₄ in biohydrolyte, anolyte effluent and other components); (B) CE, OC and TC under various operational conditions. Example note for x axis: 24/0 represents HRT and recirculation rate at 24 h and 0 mL min⁻¹, respectively.

Table C.1 Design of experiments for the MEC reactor

Input variable	Experimental run #	Anolyte flow rate (mL min ⁻¹)	Anolyte recirculation rate (mL min ⁻¹)	External resistance (ohm)
Anolyte recirculation rate (mL min ⁻¹)	1	12.5	0	1
	2	12.5	200	1
	3	12.5	500	1
	4	12.5	800	1
Flow rate (mL min ⁻¹)	5	16.7	0	1
	6	25.0	0	1
	7	16.7	800	1
	8	25.0	800	1
External resistance (ohm)	9	12.5	800	5
	10	12.5	800	10
	11	12.5	800	50
	12	12.5	800	100

Table C.2 Performance comparison on scale-up system of MEC

Scaled-up system	Anode Volume (L)	Cathode Volume (L)	Organic loading rate (kg COD m ⁻³ anode day ⁻¹)	Hydrogen productivity (m ³ m ⁻³ anode day ⁻¹)	Ref.
Single-chamber MEC	Total volume: 910 L		0.7-2	0.003-0.11	[238]
Single-chamber MEC	Total volume: 10 L		0.13-0.66	0.01-0.05	[486]
Single-chamber MEC followed by UASB ^a	Total volume: 800 L		34.2 (average)	0.023-0.069	[487]
Two-chamber MEC	88	32	0.5	0.003-0.02	[488]
Two-chamber MEC	13	2.6	0.001-0.015	0.0014 (average)	[489]

Two-chamber MEC	130	37	0.25-0.50	0.004-0.031	[490]
Present two- chamber MEC	18	1	0.2-0.4	0.005-0.006	This study

Note: ^a UASB represents for the up-flow anaerobic sludge blanket system

Appendix D for Chapter 5

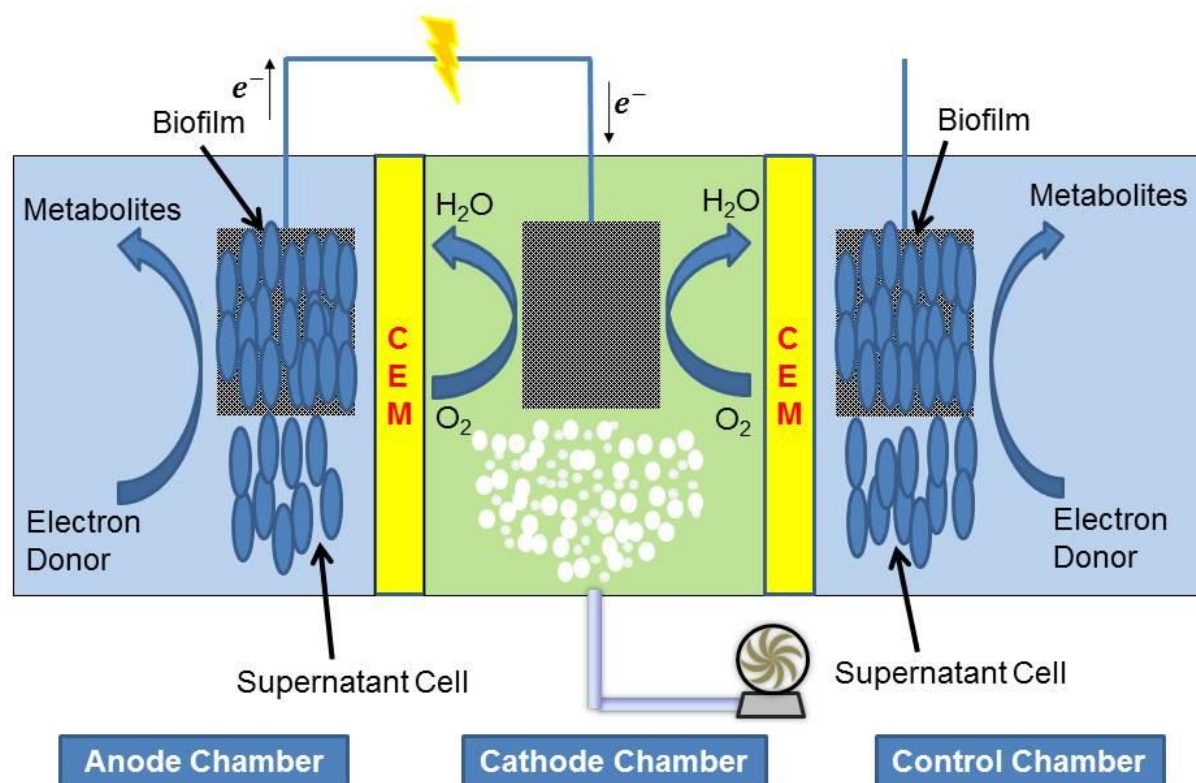


Figure D.1 Schematic of a three-chamber system consisting of an anode chamber, a cathode chamber, and a control chamber. The electrodes in the anode and the cathode chambers were connected to form an electrical circuit (functioning MFC).

Appendix E for Chapter 6

Tubular MFC setup and data measurement

A five-module tubular MFC reactor (total volume: 1.6 L) was made of cation exchange membrane (CEM, 6.8 cm diameter, Membrane International Inc., Ringwood, NJ, USA) with a length of 54 cm. Each module was about 10 cm long, containing ~320 mL of the anode volume. A single anode electrode, and a 50 cm long carbon brush (Gordon Brush Mfg. Co. Inc., Commerce, CA, USA) was shared by all the modules. The module connecting joint had sampling ports (Figure 1). Each module had its own cathode electrode, which was a piece of carbon cloth (9 cm × 22 cm, Zoltek Corporation, St. Louis, MO, USA), coated with 5 mg/cm² activated carbon powder (Thermo Fisher Scientific, USA) as a catalyst for oxygen reduction. The activated carbon powder was applied to the carbon cloth by using 15% PTFE solution as binding agent, similar to a previous study.[243] Carbon cloth wrapped the CEM tube without any heat treatment. It should be noted that the cathode electrode used here is different from a typical membrane-electrode assembly (MEA) that is used in a PEM fuel cell; because of much lower current generation in MFCs, water permeation through CEM is not sufficient for the cathode reaction and thus additional water is required to rinse the cathode (carbon cloth) for wetting. This is not considered as “cathode flooding” and does not negatively impact the MFC performance. The cathode electrode of each module was respectively connected to the shared anode electrode by copper/titanium wire across an external resistor of 4.7 Ω . The reason of choosing a low resistance of the external resistor was to significantly increase the current of each module for clear performance comparison. The MFC was operated in a continuously-fed mode at a room temperature of ~22 °C.

The voltage (V) of each module was recorded every five minutes by a digital multimeter (2700, Keithley Instruments Inc., Cleveland, OH, USA). The pH and conductivity of all catholytes, the influent and effluent anolyte samples were measured by a pH meter (Oakton Instruments, Vernon Hills, IL, USA) and a conductivity meter (Mettler-Toledo, Columbus, OH, USA).¹ The acetate concentrations of all anolyte samples were measured using HPLC (Shimadzu, Japan) with UV detector (SPD-M20A, Shimadzu, Japan) to calculate organic removal efficiency.²

During each experimental run, peristaltic pumps (BT600-2J, Langer Instruments Corp., NJ, USA) were used to control the anolyte flow rate, the catholyte circulation rate and the catholyte pump frequency (i.e., keep the circulation pump operating for one hour, or keep the pump operating for five minutes and then closed for 55 minutes in an hour).^[249] The tubular MFC reactor was placed with a small catholyte storage tank at the bottom (Figure 6.1). At the beginning of each experimental run, new catholyte would be refreshed. The peristaltic pump took the catholyte up from the tank to the top of the vertically tubular reactor to let the catholyte itself rinse along the carbon cloth down to storage tank in a batch recirculation mode. After running the newly refreshed catholyte for ten minutes to stabilize the MFC system, the data collection would start in each experiment run.

Moisture measurement and validation

Method

A 20 cm high tubular MFC reactor was made and placed vertically. The same materials and configuration were used as mentioned before (i.e., in the section of tubular MFC setup), with the exception of module segment and resistor connection. DI water was used to rinse the cathode carbon cloth for 1 min to assure complete drenching. The tubular MFC was divided to upside and downside sections to compare the moisture difference. Five pieces of delicate task wipes (4.4 inches × 8.4 inches, Kimwipes, Kimberly-Clark Professional Corp. Irving, TX, USA) were folded to wrap each module for ten seconds during the MFC operation. The same delicate task wipes mentioned in the main text were used to absorb the water to indicate the moisture. Amount of pieces of wipes folded together and wrapping period was to be determined.

Moisture Indication Equation

Water absorption weight = Total weight of wipes after wrapping – Initial wiper weight (E1)

Note: All units are gram.

Wrapping Time Period

Two one-piece wipes were wrapped tightly around the carbon cloth of the upside and the downside of MFC tube for 10 and 20 seconds respectively after complete drenching. Triplicate measurements were performed. Experimental results were shown in Figure E1. The absorbed

water weight at upside and downside was similar between 10s and 20s, and close to wipe absorption saturation. The time period of 10s was better to avoid wiper absorbing saturation for real measurement to reduce the moisture measurement inaccuracy. Thus, 10 seconds was chosen for wrapping time period.

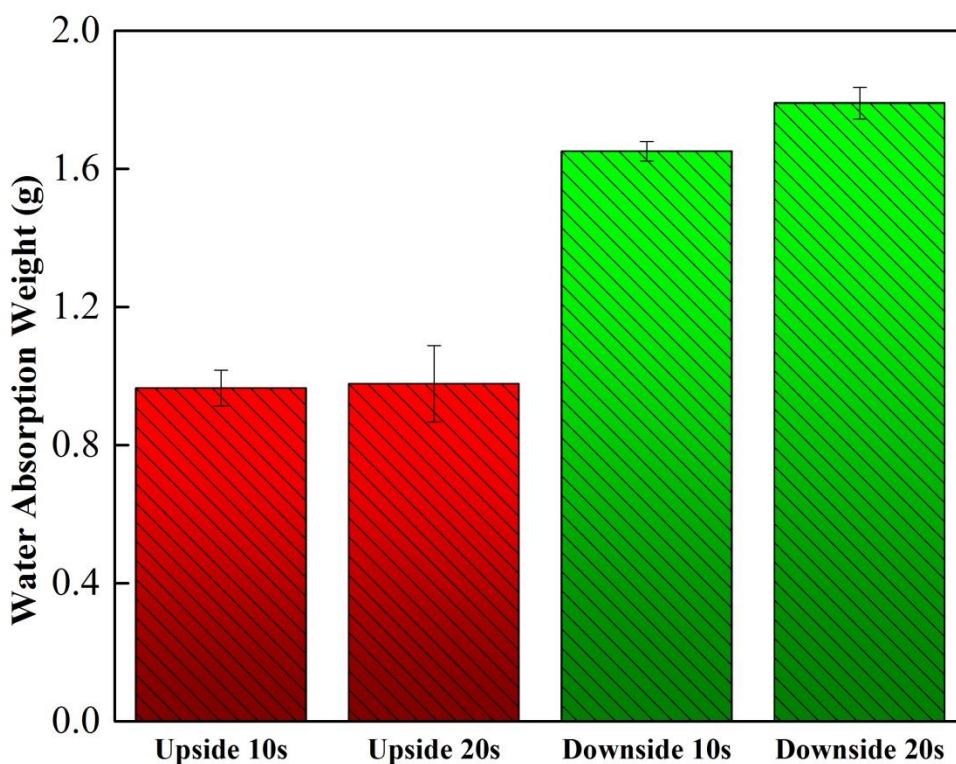


Figure E.1 Water absorbed weight of upside and downside of reactor for 10 and 20 seconds.

Amount of Paper Pieces

One, three, five, and seven pieces of wipes were folded together to see the maximum capacity of absorbed water taken from water-saturated carbon cloth respectively. Two folded wipes were wrapped tightly around cloth on the upside and the downside of MFC tube respectively for ten seconds, and recorded for their original weight and after-absorbing weight. Triplicate measurements were performed. Experimental result was shown in Figure E2.

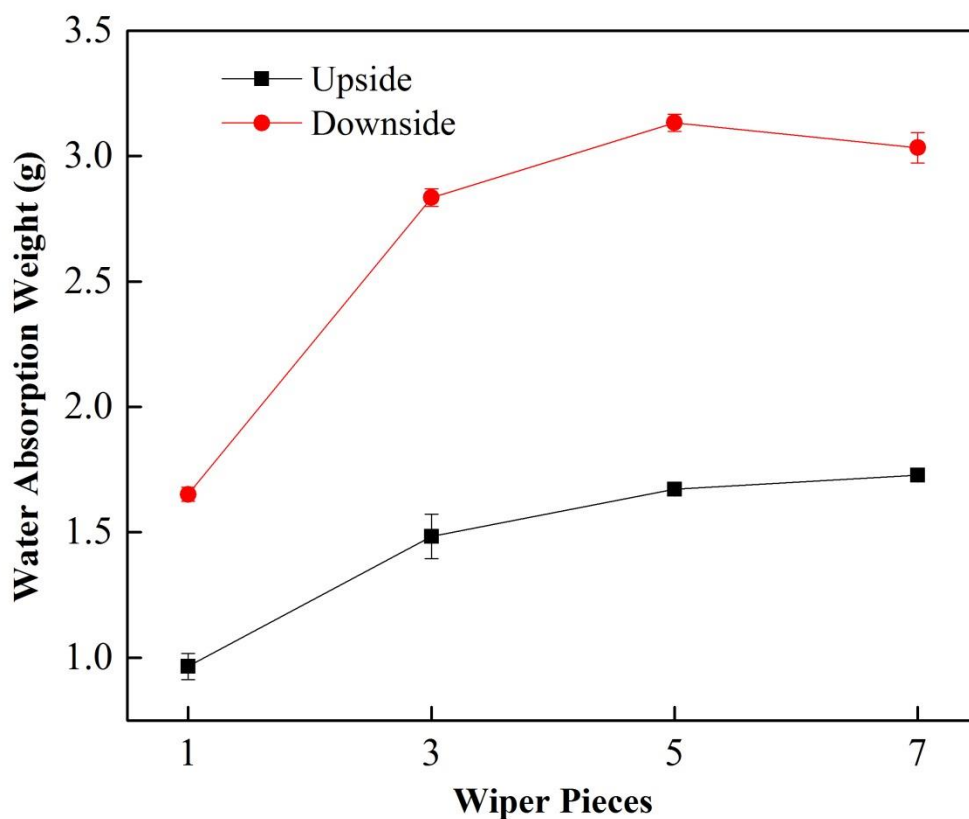


Figure E.2 Water absorbed weight using various wiper pieces (upside vs downside) wrapping for 10 seconds.

With more wipers used, water absorbed weight kept increasing from one to five pieces, but decreased or nearly reached capacity of the water absorption at seven pieces. This indicated that five pieces were enough to measure the water absorption weight in this study. Thus, five-piece wipers wrapping the carbon cloth of each module for 10 seconds was chosen for real moisture measurement.

Method Validation

Method validation was needed to see the method practicability for real measurement. Varied drying period was designed to see if the method could indicate the moisture related to the catholyte

circulation frequency and module location. After completely drenching the entire carbon cloth of the tubular reactor, it was placed in a dry place for 10 seconds, 5 minutes, 30 minutes, and 60 minutes respectively. Five-piece wipers were folded together to wrap carbon cloth (upside and downside) for 10 seconds for each of test of varied drying period. Triplicate measurements were processed. The results were shown in Figure E3.

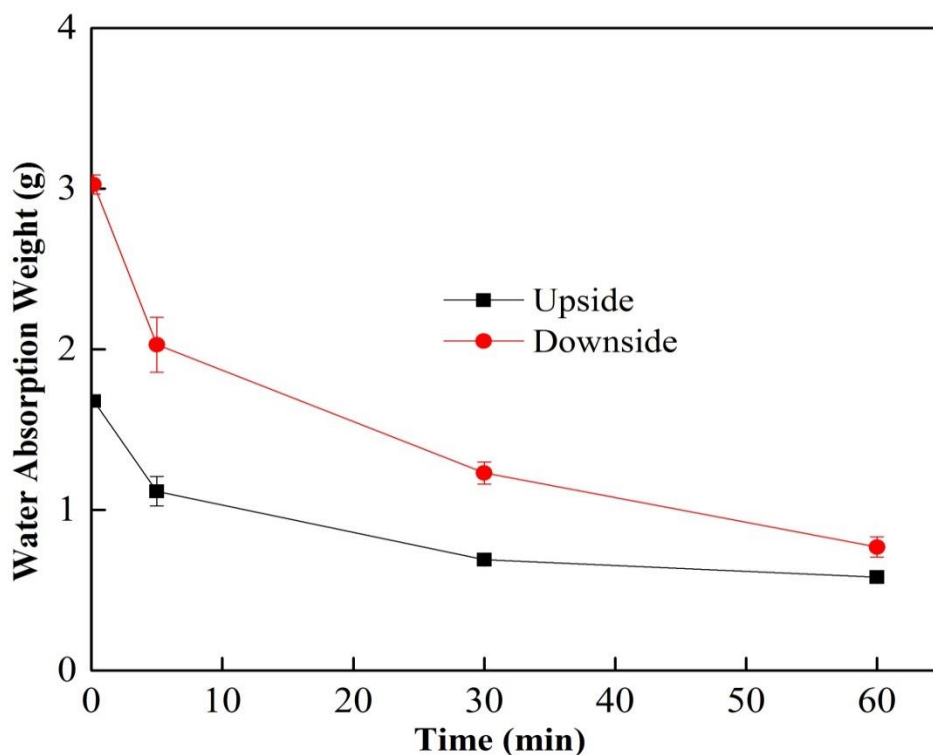


Figure E.3 Moisture measurement validation (upside and downside) under varied drying periods (i.e. 10 sec, 5 minutes, 30 minutes and 60 minutes).

The result validated that the method was able to work for actual moisture measurement related to the catholyte circulation frequency (60/0 and 5/55) and module location (M1 to M5) in this study, due to significant difference between each preliminary trial ($p < 0.05$, two sample t-test with unequal variances).

Energy calculation[29, 336]

Current (I , mA) is calculated based on Ohm's law as follows:

$$I = \frac{U}{R} * 1000 \quad (E2)$$

where R represents the external resistance (4.7 Ω).

Power density (P , W/m³) is calculated based on current (I , mA), voltage (U , V) and volume (V , m³) of each module as follows:

$$P = \frac{I*U}{V} \quad (E3)$$

where V represents the volume of each module (3.2*10⁻⁴ m³)

Power density of entire MFC reactor (P_T , W/m³) is the sum of power density of each module:

$$P_T = \sum(P1 + P2 + P3 + P4 + P5) \quad (E4)$$

Accumulated NER based on flowing volume (NER_V , kWh/m³) and on acetate removal (NER_A , kWh/kg sodium acetate) of each module every five minutes is shown as below:

$$NER_V = \frac{I*U}{F*60} \quad (E5)$$

$$NER_A = \frac{NER_V*12.2}{\Delta_{sodium\ acetate}} \quad (E6)$$

where F is the anolyte flow rate (2 mL/min or 3mL/min); $\Delta_{sodium\ acetate}$ is the amount of acetate consumed through a module (mM).

In most cases, accumulated NER of each module measured every five minutes is presented as an example in Figure 6.2. The NER of entire system is obtained by summing the NER of all five modules.

Accumulated NER of the entire system every five minutes is obtained as follows:

$$NER_{VT} = \sum NER_V(M1\ to\ M5), \quad (E7)$$

$$NER_{AT} = \sum NER_A(M1\ to\ M5), \quad (E8)$$

where NER_{VT} is the accumulated NER of entire system based on flowing volume (kWh/m³) every five minutes; and NER_{AT} is the accumulated NER of entire system based on acetate removal (kWh/kg sodium acetate) every five minutes. M1 to M5 means from module 1 to module 5.

Averaged accumulated NER of the entire system every five minutes through an entire run (three-hour period) is obtained as follows:

$$NER_{VTA} = \frac{\sum(NER_{VT1} + NER_{VT2} + \dots + NER_{VT36})}{36} \quad (E9)$$

$$NER_{ATA} = \frac{\sum(NER_{AT1} + NER_{AT2} + \dots + NER_{AT36})}{36} \quad (E10)$$

where NER_{VTA} is the average of five-minute NER of entire system based on flowing volume (kWh/m^3) through a three-hour Run; NER_{ATA} is the average of five-minute NER of entire system based on acetate removal (kWh/kg sodium acetate) through a three-hour Run.

Equations for organic loading rate (OLR, $\text{kg}/(\text{m}^3 \cdot \text{d})$) and hydraulic retention time (HRT, h) in this study are listed as follows:

$$OLR = 0.00144 * \frac{\text{anolyte flow rate} * \text{anolyte influent sodium acetate concentration}}{\text{anode total volume}} \quad (E11)$$

$$HRT = 16.67 * \frac{\text{anode volume}}{\text{anolyte flow rate}} \quad (E12)$$

where units for anolyte flow rate, anolyte influent sodium acetate concentration and anode volume are mL/min , mg/L and L , respectively. Anode volume is 1.6 L .

Two-sample test

In this section, the NER and power density are evaluated with two-sample t-test to check if they are statistically different under different design factors combinations. Specifically, Runs 13 and 15, Runs 13 and 14, Runs 15 and 16, Runs 1 and 11, Runs 1 and 7, and Runs 11 and 15 are compared with two-sample t-test. In each run, there are 36 measurements within a 3-hour operating period, so the sample size for the test is 36. The test statistics is,

$$t = \frac{\bar{x} - \bar{y}}{\sqrt{\frac{s_x^2}{n} + \frac{s_y^2}{n}}} \quad (E13)$$

where $\bar{x}$ and $\bar{y}$ are the sample means of the two Runs under comparison, s_x and s_y are the sample standard deviations, and n is the sample size. The p-value is used to evaluate whether the two runs are significantly different. The smaller the p-value, the more confidence one can conclude that the two runs are different. Normally, a p-value of less than 0.05 is used to conclude the significant difference. The p-values for all the above pairs of tests for all responses are significant, i.e., the NER and power density are different under different design factor combinations.

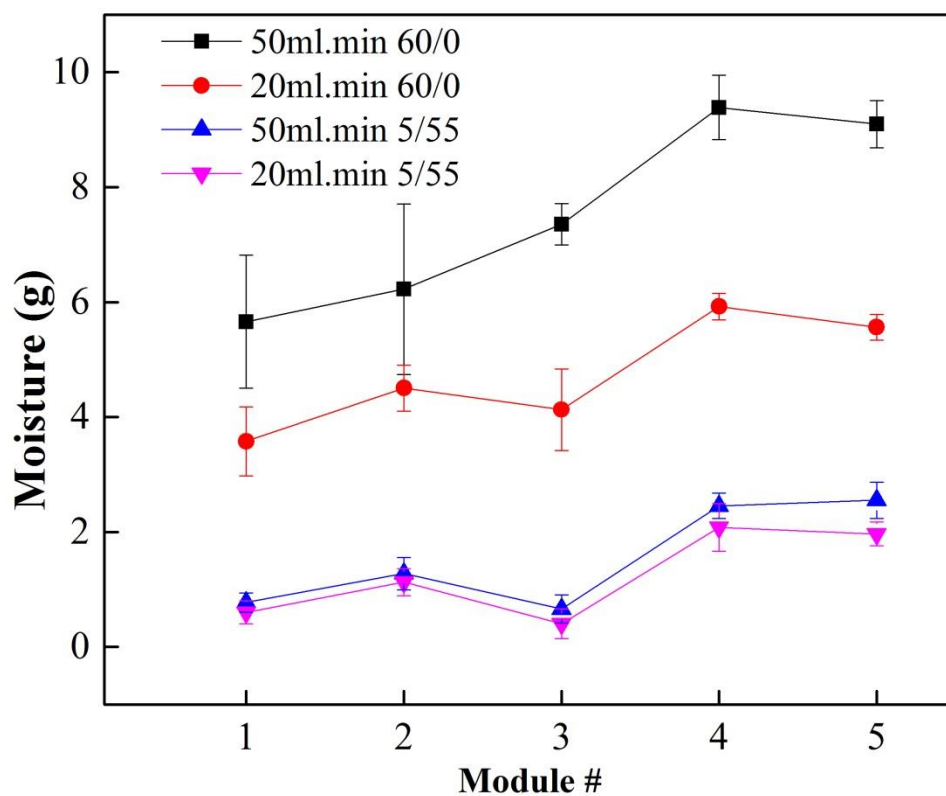


Figure E.4 Moisture measurements on five modules under varied cathode operating conditions. Note: Each data point shown is the average moisture over the 12 measurement data collected from three one-hour intervals (i.e., at the end of 1st, 2nd and 3rd hour) over four runs of varied cathode operating conditions.

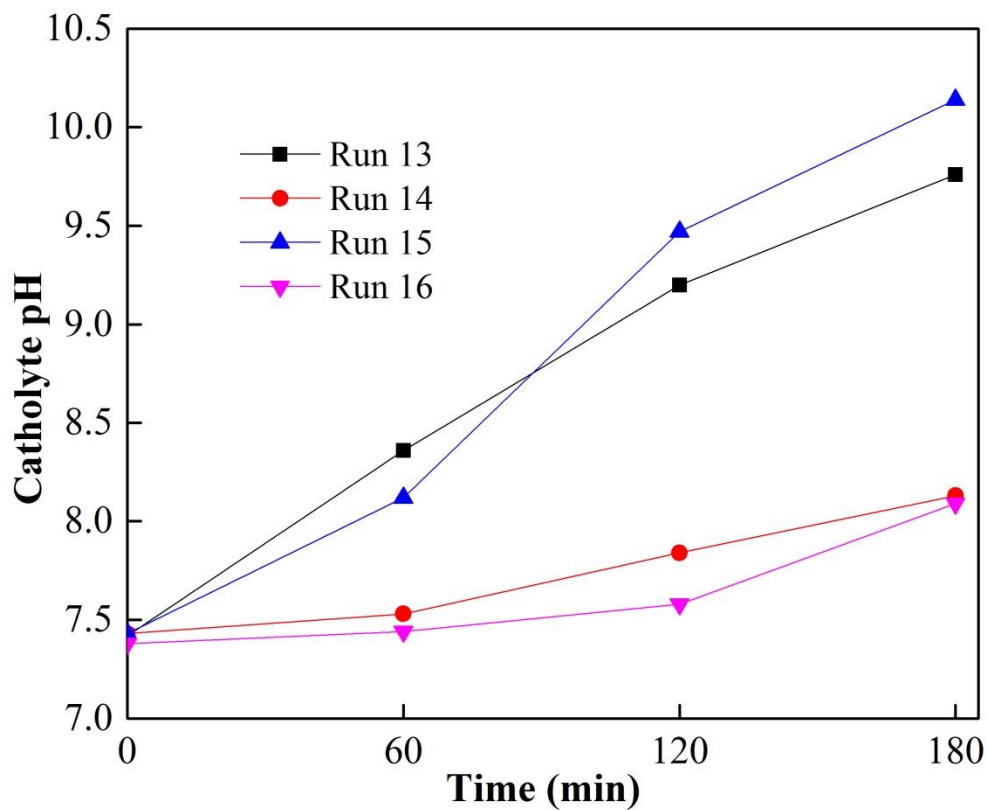


Figure E.5 Catholyte pH change in the three-hour experiment of Runs 13 to 16. Note: Run 13: 20mL/min 60/0 + 500mg/L 3mL/min; Run 14: 20mL/min 5/55 + 500mg/L 3mL/min; Run 15: 50mL/min 60/0 + 500mg/L 3mL/min; Run 16: 50mL/min 5/55 + 500mg/L 3mL/min.

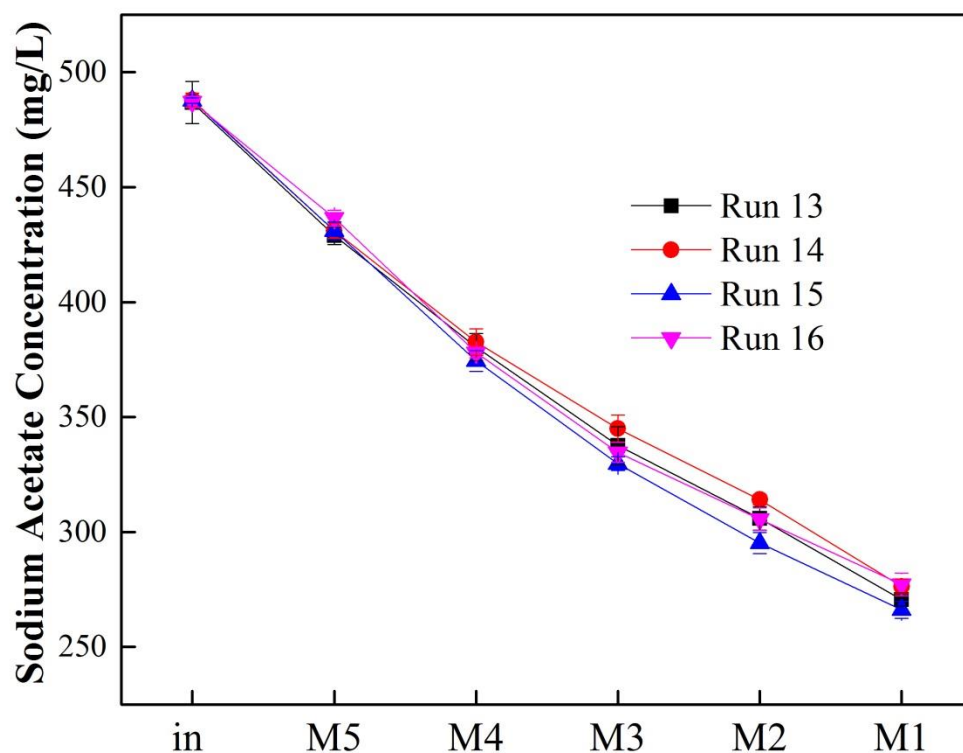


Figure E.6 Sodium acetate concentration in all modules of Runs 13 to 16. Note: Run 13: 20mL/min 60/0 + 500mg/L 3mL/min; Run 14: 20mL/min 5/55 + 500mg/L 3mL/min; Run 15: 50mL/min 60/0 + 500mg/L 3mL/min; Run 16: 50mL/min 5/55 + 500mg/L 3mL/min. “in” in x-axis represents influent.

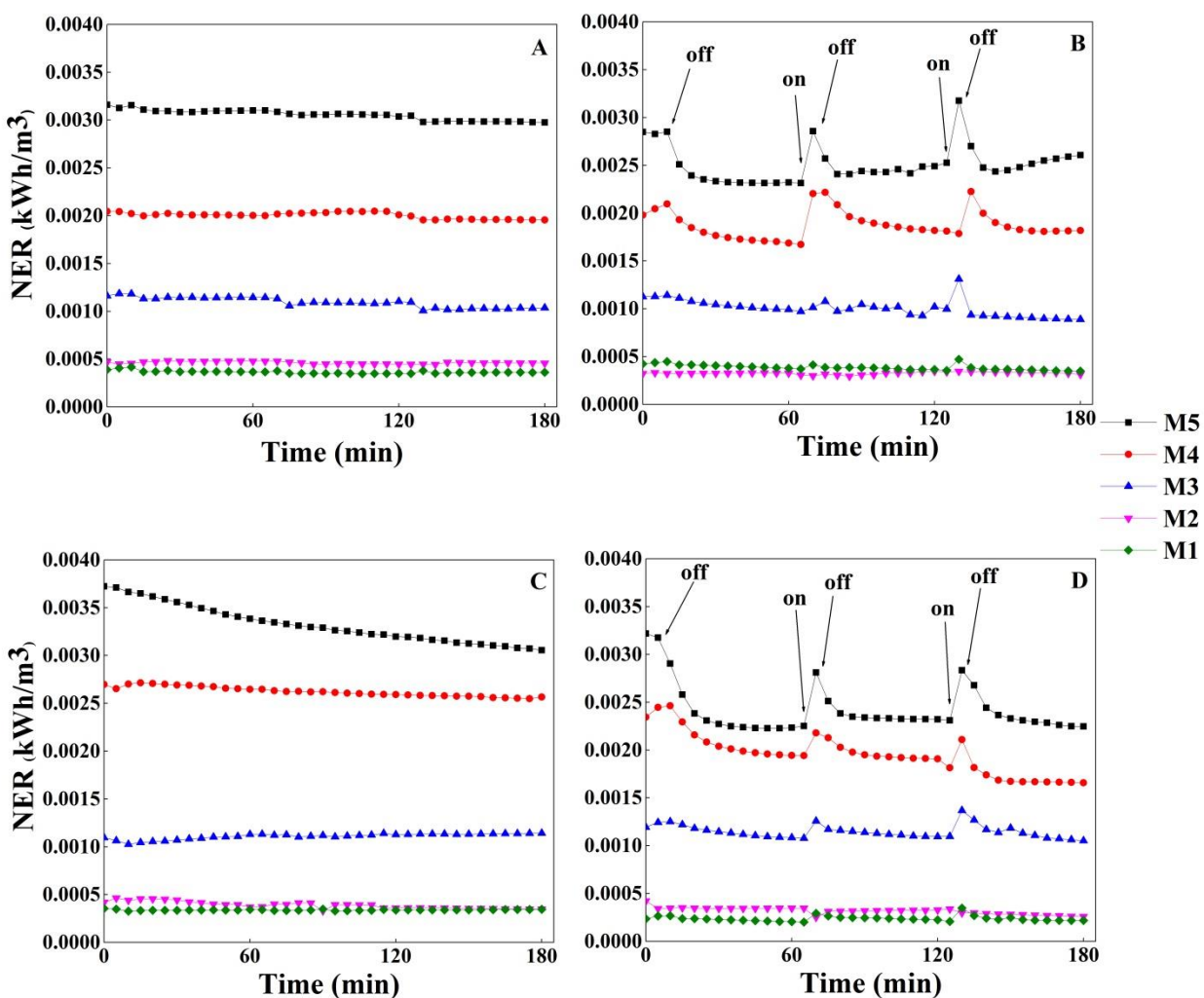


Figure E.7 NER (kWh/m³) of all modules under the same anode operating conditions. (A) Run 13: 20mL/min 60/0 + 500mg/L 3mL/min; (B) Run 14: 20mL/min 5/55 + 500mg/L 3mL/min; (C) Run 15: 50mL/min 60/0 + 500mg/L 3mL/min; (D) Run 16: 50mL/min 5/55 + 500mg/L 3mL/min. Note: “on” means “turn the catholyte pump on”; “off” means “turn the catholyte pump off”.

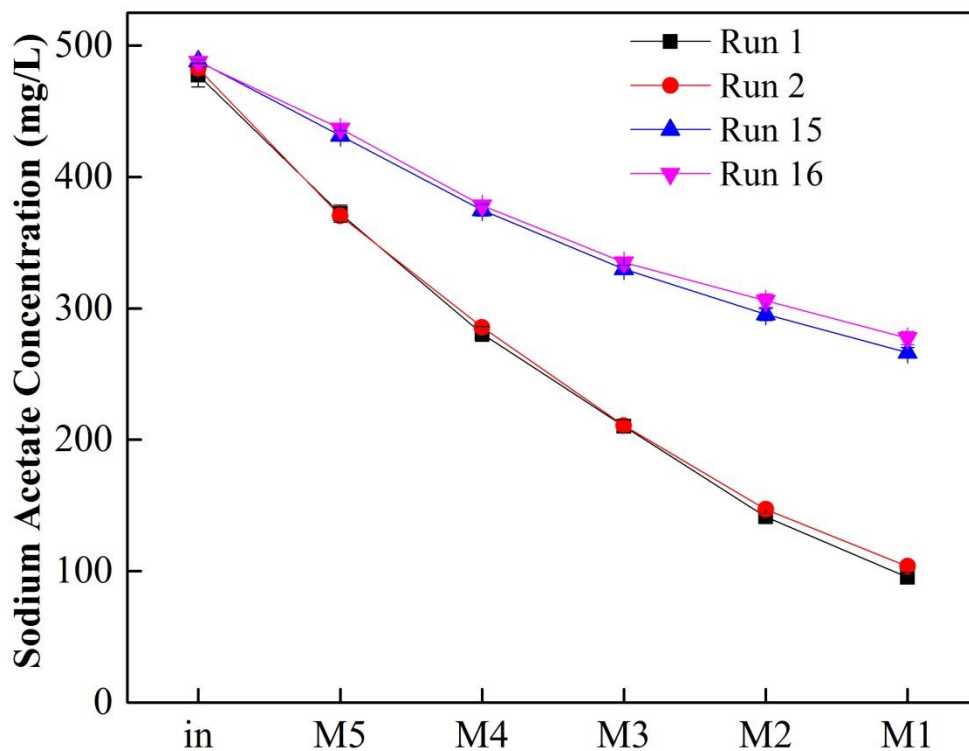


Figure E.8 Respective acetate concentration in all modules under various anolyte flow rate and cathode moisture. Note: Run 1: 50mL/min 60/0 + 500mg/L 2mL/min; Run 2: 50mL/min 5/55 + 500mg/L 2mL/min; Run 15: 50mL/min 60/0 + 500mg/L 3mL/min; Run 16: 50mL/min 5/55 + 500mg/L 3mL/min. “in” in x-axis represents influent.

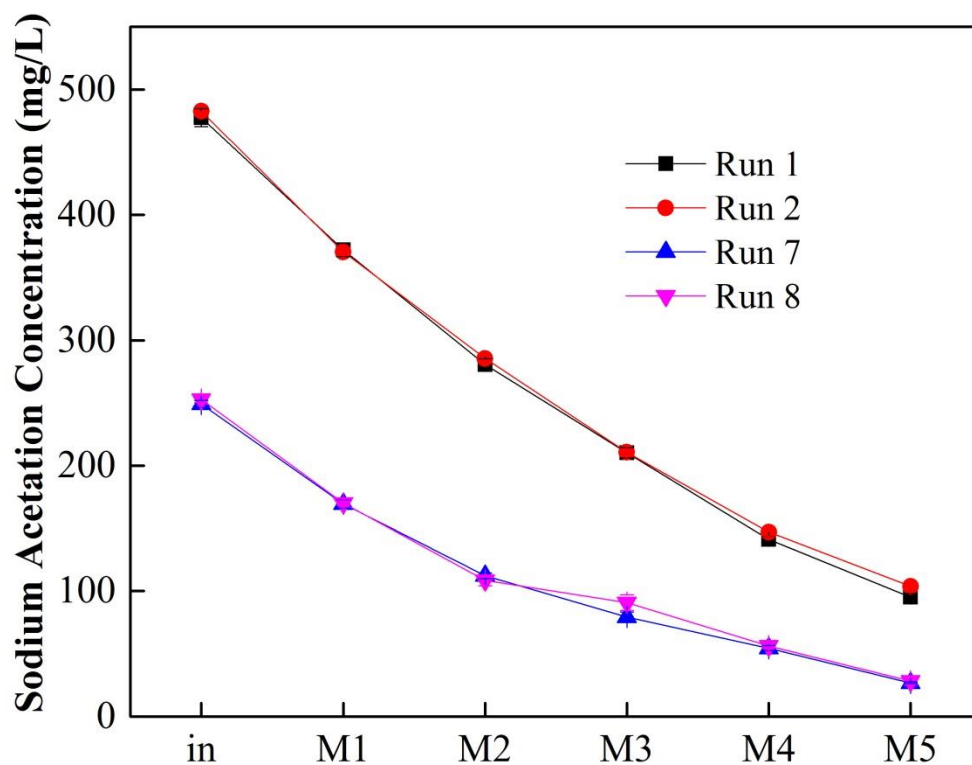


Figure E.9 Respective acetate concentration in all modules under varied influent sodium acetate concentration and cathode moisture. Note: Run 1: 50mL/min 60/0 + 500mg/L 2mL/min; Run 2: 50mL/min 5/55 + 500mg/L 2mL/min; Run 7: 50mL/min 60/0 + 250mg/L 2mL/min; Run 8: 50mL/min 5/55 + 250mg/L 2mL/min. “in” in x-axis represents influent.

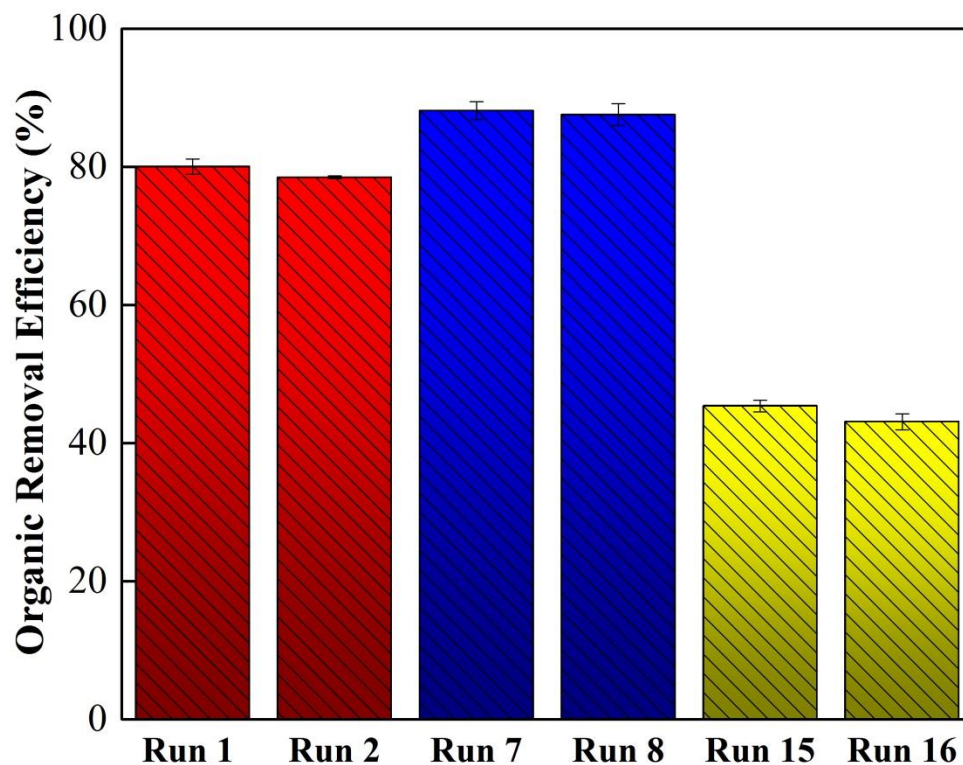


Figure E.10 Effect of varied operating conditions on organic removal efficiency. Note: Run 1: 50mL/min 60/0 + 500mg/L 2mL/min; Run 2: 50mL/min 5/55 + 500mg/L 2mL/min; Run 7: 50mL/min 60/0 + 250mg/L 2mL/min; Run 8: 50mL/min 5/55 + 250mg/L 2mL/min; Run 15: 50mL/min 60/0 + 500mg/L 3mL/min; Run 16: 50mL/min 5/55 + 500mg/L 3mL/min).

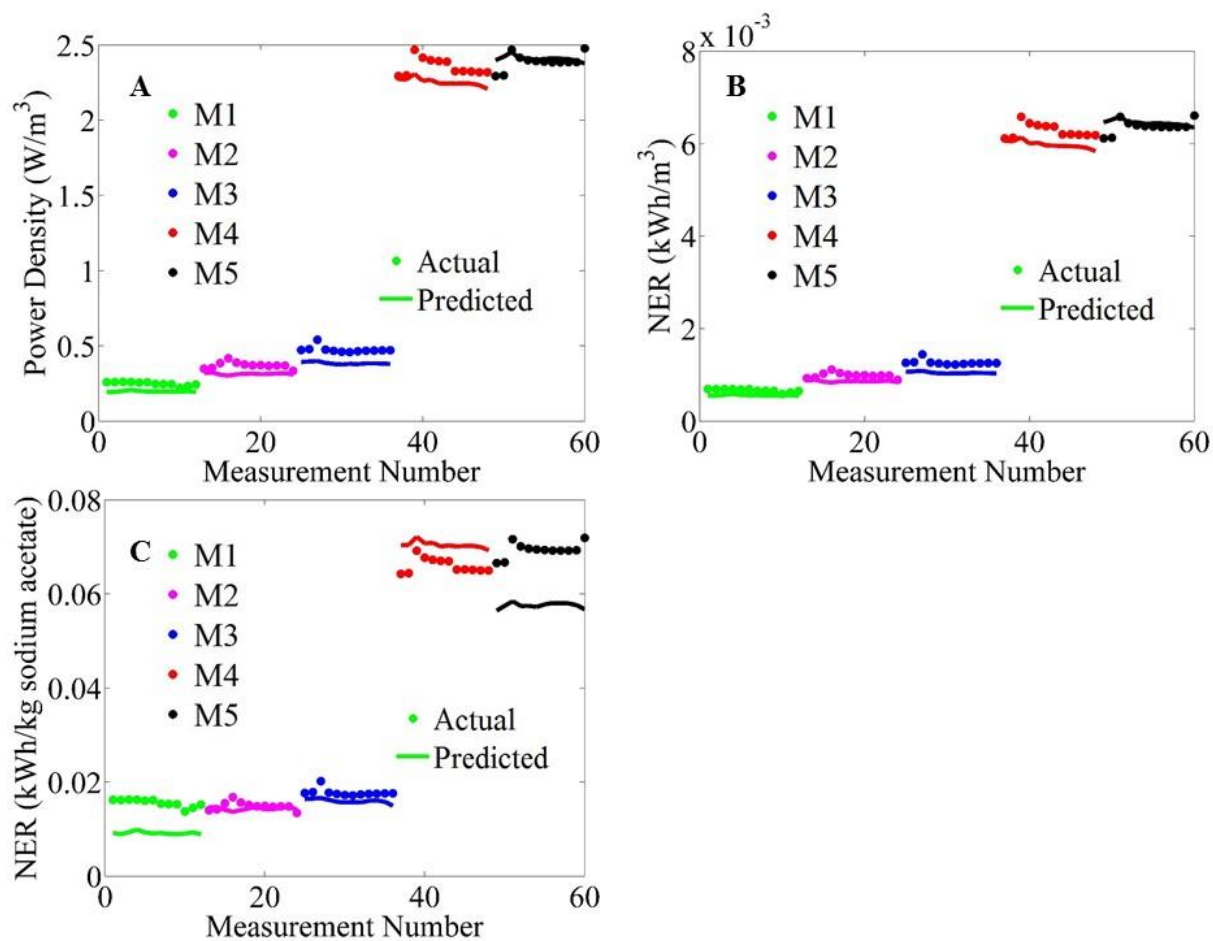


Figure E.11 Actual versus predicted (A) power density, (B) NER (kWh/m^3) and (C) NER (kWh/kg sodium acetate) from MLM.

Catholyte conductivity x_{16}				
Catholyte pH x_{15}				
Organic at M5 x_{14}				
Organic at M4 x_{13}				
Organic at M3 x_{12}				
Organic at M2 x_{11}				
Organic at M1 x_{10}				
Moisture at M5 x_9				
Moisture at M4 x_8				
Moisture at M3 x_7				
Moisture at M2 x_6				
Moisture at M1 x_5				
Anolyte flow rate x_4				
Anolyte acetate concentration x_3				
Catholyte circulation rate x_2				
Catholyte pump frequency x_1				
	Current (mA)	Power density (W/m ³)	NER (kWh/m ³)	NER (kWh/kg sodium acetate)
				Organic remove efficiency (%)

Figure E.12 Significant variables for MLM (significant variables filled with black).

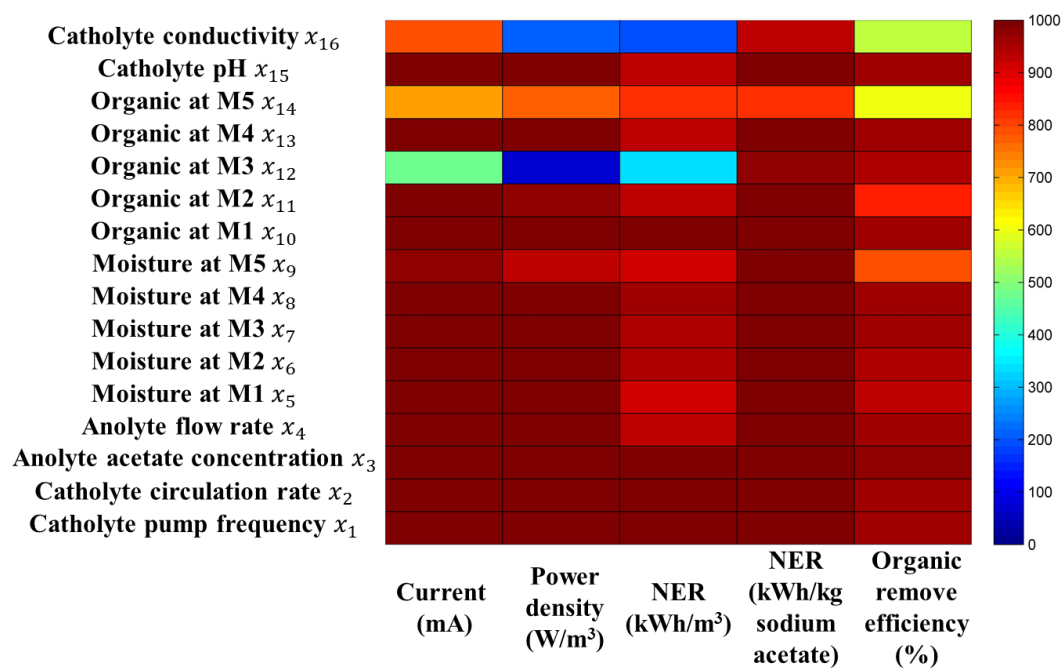


Figure E.13 Variable Selection Over 1000 Replications

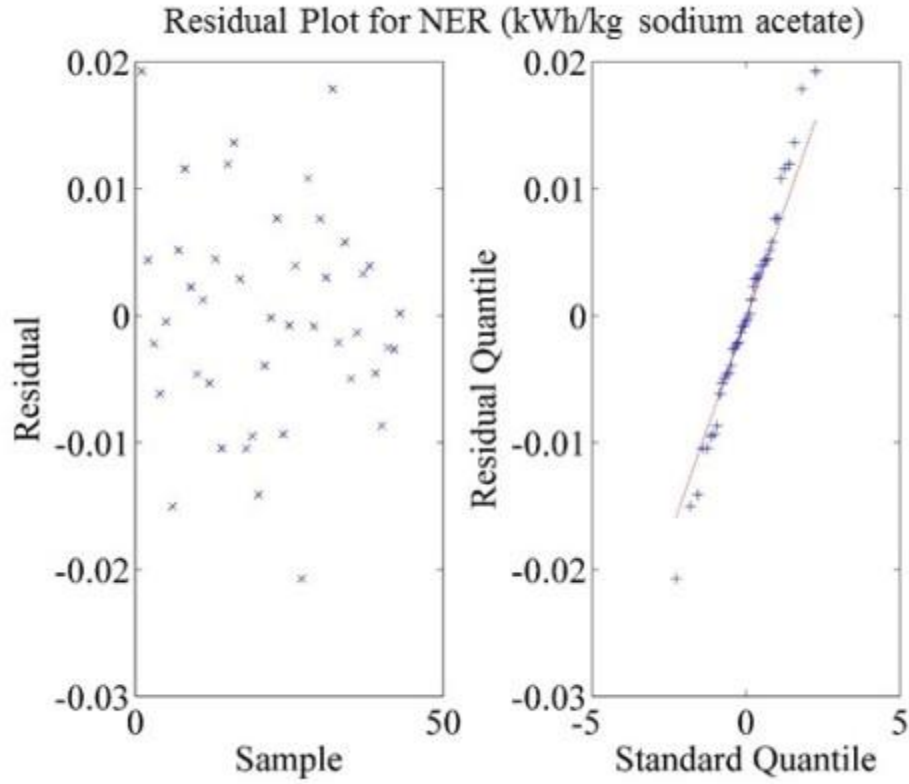


Figure E.14 Residual plot for NER (kWh/kg sodium acetate) at M5 for MLM.

From Figure E14, the residuals are approximately identically, independently and normally distributed. Therefore, the model assumptions are not violated. Specifically, the left figure shows that the residuals are of the same scale, and there is no abnormal pattern. From the right figure, the residuals are normally distributed.

Root Mean Squared Error [366]

Root mean squared error (RMSE) is adopted as model performance evaluation criterion:[366]

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^N \sum_{j=1}^q (y_{i,j} - \hat{y}_{i,j})^2}{N \times q}} \quad (\text{E14})$$

where N is the total number of samples, and q is the number of elements in a certain output. $y_{i,j}$ and $\hat{y}_{i,j}$ represent experimental data and model predicted value for the i -th sample at the j -th element, respectively. The maximum value of experimental data is used to normalize the error.

The RMSEs for the testing errors in MLM and separate modeling are summarized in Table E1 and Table E2, respectively. The separate modeling uses multiple linear regressions with stepwise variable selection. From the tables, the MLM has smaller testing error (in percentage) than separate regression models with stepwise variable selection.

Table E.1 MLM testing performance summary.

CV Fold	Current RMSE	Power Density RMSE	NER (kWh/m³) RMSE	NER (kWh/kg sodium acetate) RMSE	Organic Removal Efficiency RMSE
CV 1	3.90%	4.96%	4.46%	10.20%	2.11%
CV 2	3.53%	3.96%	4.19%	11.17%	3.74%
CV 3	4.68%	4.83%	4.52%	8.34%	2.38%
CV 4	5.33%	6.66%	5.58%	7.25%	1.79%
CV 5	4.90%	5.78%	5.33%	11.99%	16.27%
CV 6	3.07%	4.36%	3.30%	8.29%	1.91%
CV 7	3.64%	4.91%	4.84%	8.92%	1.46%
CV 8	4.36%	6.21%	5.12%	7.61%	1.55%
CV 9	3.29%	3.62%	2.88%	9.98%	2.44%
CV 10	4.48%	4.95%	3.96%	10.10%	2.29%
Mean	4.12%	5.02%	4.42%	9.38%	3.59%

Table E.2 Summary of the separate modeling testing performance.

CV Fold	Current RMSE	Power Density RMSE	NER (kWh/m³) RMSE	NER (kWh/kg sodium acetate) RMSE	Organic Removal Efficiency RMSE
CV 1	55.04%	88.47%	89.24%	154.69%	22.33%
CV 2	35.20%	43.93%	53.27%	165.10%	33.32%
CV 3	34.03%	47.03%	36.28%	147.34%	21.20%
CV 4	44.18%	57.45%	42.85%	123.70%	27.93%

CV 5	47.35%	61.06%	50.10%	165.24%	31.09%
CV 6	44.73%	59.73%	59.44%	248.82%	31.78%
CV 7	51.79%	58.84%	55.26%	275.67%	38.78%
CV 8	39.89%	61.55%	65.54%	262.96%	19.96%
CV 9	39.91%	45.63%	57.77%	115.92%	24.01%
CV 10	43.98%	63.45%	56.15%	127.84%	39.49%
Mean	43.61%	58.71%	56.59%	178.73%	28.99%

p-Value

The significant variables identified from MLM (Figure E12) are used to refit a linear regression. The p-values of the model coefficients are calculated from a t-test, and provided in Table E3. The smaller a p-value, the more important a variable is.

Table E.3 Variable importance (p-value) for system overall performance.

	NER (kWh/kg Sodium Acetate)	NER (kWh/m ³)	Organic Removal Efficiency
Catholyte pump frequency (x_1)	0.0000	0.0000	0.2310
Catholyte circulation rate (x_2)	0.2790	0.0986	0.1350
Anolyte influent sodium acetate concentration (x_3)	0.0000	0.0000	0.0000
Anolyte flow rate (x_4)	0.0000	0.0000	0.0000
Moisture at M1 (x_5)	0.0207	0.0127	0.4410
Moisture at M2 (x_6)	0.6350	0.1290	0.5990
Moisture at M3 (x_7)	0.0147	0.1970	0.0018
Moisture at M4 (x_8)	0.0035	0.0000	0.0007
Moisture at M5 (x_9)	0.0571	0.0027	0.0531
Organic at M1 (x_{10})	0.0000	0.0000	0.0000
Organic at M2 (x_{11})	0.4810	0.2220	0.7180
Organic at M3 (x_{12})	0.0020	NA	0.7730

Organic at M4 (x_{13})	0.0103	0.4930	0.0115
Organic at M5 (x_{14})	0.1190	0.2480	0.0196
Catholyte pH (x_{15})	0.2350	0.7390	0.0251
Catholyte conductivity (x_{16})	0.1690	NA	0.4350

Note: NA means the corresponding variable is not selected in MLM, and is not used in refitting the linear regression.

Appendix F for Chapter 7

Internal resistance measurement

Multimeter (Extech EX540, Extech Instrument) was used to measure voltage under close circuit (V_1), with two cables ports to connect anode and cathode respectively. The current was measured as voltage divided by external resistance ($R_{ext} = 2 \text{ ohm}$). Then, the voltage was interrupted by disconnecting the external resistance to the electrode, and the voltage after interruption was recorded (V_2). The rationality to use current interruption was that the MFC performance would become stable for current generation after sufficient operation time. The voltage had immediately steep rise after converting the close circuit to open circuit, and the steep voltage rise represented the voltage posed on the internal resistance (mainly ohmic loss) [383, 491-493]. The internal resistance was measured by the calculation shown as the following equations [383, 494-496]:

$$R_{int} = \frac{V_2 - V_1}{V_1} * R_{ext} \quad (F1)$$

Table F.1 Experimental design table

Region	Run #	Designed Factor Values		
		Anode Flow Rate (x_1) (mL min ⁻¹)	Acetate Concentration (x_2) (g L ⁻¹)	Catholyte Pump Flow Rate (x_3) (mL min ⁻¹)
Normal Region (Full Factorial Design)	1	2.00	0.32	2.00
	2	3.00	0.32	2.00
	3	3.00	0.64	2.00
	4	2.00	0.64	2.00
	5	2.00	0.32	50.00
	6	3.00	0.32	50.00
	7	2.00	0.64	50.00
	8	3.00	0.64	50.00
Normal Region (Space Filling Design)	9	2.45	0.32	39.16
	10	2.06	0.38	25.23
	11	2.29	0.36	22.13
	12	2.03	0.41	23.68
	13	2.65	0.33	17.48
	14	2.74	0.34	37.61
	15	2.58	0.35	5.10
	16	2.61	0.37	3.55
	17	2.16	0.46	50.00
	18	2.52	0.39	20.58
	19	2.10	0.50	19.03

	20	2.00	0.53	36.06
	21	2.68	0.40	42.26
	22	2.19	0.52	43.81
	23	2.23	0.49	6.65
	24	2.13	0.54	11.29
	25	2.87	0.42	40.71
	26	2.81	0.45	8.19
	27	2.32	0.56	9.74
	28	3.00	0.43	15.94
	29	2.77	0.48	45.35
	30	2.90	0.47	32.97
	31	2.26	0.62	14.39
	32	2.39	0.59	48.45
	33	2.48	0.57	31.42
	34	2.42	0.61	46.90
	35	2.35	0.63	34.52
	36	2.84	0.51	2.00
	37	2.71	0.60	29.87
	38	2.97	0.55	28.32
	39	2.55	0.64	12.84
	40	2.94	0.58	26.77
Extreme Region 1 (Full Factorial Design)	41	1.50	0.26	1.50
	42	4.00	0.26	1.50
	43	4.00	0.26	75.00
	44	1.50	0.26	75.00
	45	1.50	0.83	75.00
	46	4.00	0.83	75.00
	47	4.00	0.83	1.50
	48	1.50	0.83	1.50
Extreme Region 2 (Full Factorial Design)	49	1.00	1.03	1.00
	50	5.00	1.03	1.00
	51	5.00	1.03	100.00
	52	1.00	1.03	100.00
	53	1.00	0.19	100.00
	54	1.00	0.19	1.00
	55	5.00	0.19	1.00
	56	5.00	0.19	100.00

Engineering model for MFC

The MFC engineering model is used to simulate the dynamic performance of anode and cathode together. For anode part, the mass balance of the growth of microorganisms, substrate degradation, intracellular redox mediator and electrochemical reaction in the anode are considered, following

the assumption that the microbial growth follows multiplicative Monod kinetics [150, 166]. The growth condition of anodophilic and methanogenic bacteria is simulated based on the following equations Eqs. (F2)-(F3):

$$\frac{dx_a}{dt} = u_a \cdot x_a - k_{da} \cdot x_a - \frac{\varepsilon_a \cdot F}{V_a} \cdot x_a \quad (F2)$$

$$\frac{dx_m}{dt} = u_m \cdot x_m - k_{dm} \cdot x_m - \frac{\varepsilon_m \cdot F}{V_a} \cdot x_a \quad (F3)$$

where x_a and x_m represent concentration of anodophilic and methanogenic bacteria in anode (mg L⁻¹), respectively; u_a and u_m represent specific growth rate of anodophilic and methanogenic microorganisms (day⁻¹), respectively; k_{da} and k_{dm} represent decay rate of anodophilic and methanogenic bacteria (day⁻¹), respectively; ε_a and ε_m represent biofilm retention constant for anodophilic and methanogenic bacteria (dimensionless), respectively; V_a is the anode volume (L); F is the flow rate (L day⁻¹).

The assisting equations to complete ODEs above Eqs. (F2)-(F3), and the parameter derivation are listed below, based on the previous work [166]

$$\varepsilon_a = \frac{1 + \tanh(k_x(x_a + x_m - x_{max-a}))}{2} \quad (F4)$$

$$k_{da} = 0.02 \cdot u_{max-a} \quad (F5)$$

$$u_a = \frac{u_{max-a} \cdot S_a}{k_{sa} + S_a} \cdot \frac{MOX}{k_m + MOX} \quad (F6)$$

$$\varepsilon_m = \frac{1 + \tanh(k_x(x_a + x_m - x_{max-m}))}{2} \quad (F7)$$

$$k_{dm} = 0.02 \cdot u_{max-m} \quad (F8)$$

$$u_m = \frac{u_{max-m} \cdot S_a}{k_{sm} + S_a} \quad (F9)$$

where k_x is the steepness factor for anodophilic and methanogenic microorganism (L mg⁻¹); x_{max-a} and x_{max-m} represent the maximum attainable concentrations for anodophilic and methanogenic bacteria (mg L⁻¹), respectively; u_{max-a} and u_{max-m} represent the maximum specific growth rate of anodophilic and methanogenic microorganisms (day⁻¹), respectively; k_{sa} and k_{sm} represent the half-rate constant for anodophilic and methanogenic microorganisms (mg

L^{-1}); S_a is the substrate concentration in anode ($mg\ L^{-1}$) (sodium acetate in this study); M_{OX} is fraction of oxidized mediator versus total mediator fraction per microorganisms (dimensionless); k_m is the half-rate constant of mediator (dimensionless).

The intracellular mediator is assumed to be generated from anodophilic bacteria as transporter for the electrons, to aid electron transportation from substrate degradation to the anode electrode based on previous finding [166]. The oxidized mediator (M_{OX}) is the mediator already releasing electrons towards the anode electrode, and then the oxidized mediator comes back to accept electrons from anodophilic bacteria to be reduced to reduced mediator (M_{RED}), forming an electron transporting cycle to realize current generation. Oxidized mediator (M_{OX}) is calculated in the ODE for dynamic behavior of mediator fraction Eq. (F10)-(F11), based on previous work [166]:

$$\frac{dM_{OX}}{dt} = -Y_M \cdot q_a + \frac{1000 \cdot 86400 \cdot \gamma \cdot I}{n_e \cdot F_a \cdot V_a \cdot x_a} \quad (F10)$$

$$k_m = 0.02 \cdot m_{total} \quad (F11)$$

where Y_M is the mediator yield (dimensionless); $n_e = 2$ [166][166][166][166][166][166][9][9]; $\gamma = 663400\ mg\ mole^{-1}$; I represents the current (A); F_a is the Faraday constant = $96485\ C\ mol^{-1}$; $m_{total} = 0.05$ per microorganisms (dimensionless) [166]; q_a is the substrate degradation rate ($mg\ day^{-1}$) by anodophilic bacteria calculated in the following equation Eq. (F12), and the methanogenic substrate consumption rate q_m ($mg\ day^{-1}$) is calculated in Eq. (F13):

$$q_a = \frac{q_{max-a} \cdot S_a}{k_{sa} + S_a} \cdot \frac{M_{OX}}{k_m + M_{OX}} \quad (F12)$$

$$q_m = \frac{q_{max-m} \cdot S_a}{k_{sm} + S_a} \quad (F13)$$

where q_{max-a} and q_{max-m} represent the maximum anodophilic and methanogenic COD reaction rate (day^{-1}); k_{sa} and k_{sm} represent the half-rate constant for anodophilic microorganisms ($mg\ L^{-1}$). The ODE for substrate degradation in anode can be listed below based on the above parameter calculation.

$$\frac{dS_a}{dt} = -q_a \cdot x_a - q_m \cdot x_m + \frac{F}{V_a} (S_{in} - S_a) \quad (F14)$$

where S_{in} and S_a are the acetate concentration in the anode influent and effluent ($mg\ L^{-1}$), respectively. The acetate concentration in the effluent of each MFC module would be the influent concentration for the following MFC module.

For the current generation of each MFC module, the basic equation is to consider electrode overpotential and internal resistance to deduct the available voltage out of the open circuit voltage, based on previous work [166]:

$$I \cdot R_{ext} = V_{OC} - OP_{anode} - OP_{cathode} - OP_{conc} - I \cdot R_{int} \quad (S15)$$

where R_{ext} is external resistance ($R_{ext} = 2 \Omega$ in this study); V_{OC} is the open circuit voltage (V); OP_{anode} and $OP_{cathode}$ represent the overpotential (V) of anode and cathode, respectively; OP_{conc} is the concentration overpotential (V).

It was assumed that the anode overpotential could be neglected because sufficient buffer solution was applied in the anolyte to eliminate the interfering effect of pH (most influential parameter on anode potential) [166]. The cathode overpotential was assumed to be constant with little impact on internal resistance under various operating conditions, because the of the passive oxygenation on the cathode material with sufficient oxygen to supply, and high concentration of activated carbon was coated on the carbon cloth to guarantee the cathodic oxygen reduction reaction. Thus, OP_{anode} and $OP_{cathode}$ can be assumed to be zero in this study. The following equations were applied to assist the calculation of V_{OC} , OP_{conc} :

$$V_{OC} = V_{min} + (V_{max} - V_{min}) \cdot e^{-\frac{1}{k_r \cdot x_a}} \quad (F16)$$

$$OP_{conc} = \frac{R \cdot T}{F_a} \ln \left(\frac{M_{total}}{M_{red}} \right) \quad (F17)$$

$$m_{total} = M_{red} + M_{ox} \quad (F18)$$

where V_{min} and V_{max} represents the lowest and maximum circuit voltage (V), respectively; k_r is constant determining the speed of internal resistance responding to the change in microorganism concentration ($L \cdot mg^{-1}$), which represents the response speed of the impact of changing internal resistance on voltage once the microorganism concentration has dynamic change; R is the ideal gas constant ($8.314 J \cdot K^{-1} \cdot mol^{-1}$); T is the temperature (298.15 K).

The current interruption method is applied to measure the internal resistance for each MFC module at the end of 1st- and 3rd-hour during a 3-hour experimental run. At the same time, the Gaussian Process (GP) regression model is used to model the relationship among explanatory variables (catholyte pumping rate, influent acetate concentration, and anolyte flow rate), their two-way

interactions and the dependent variable (R_{int}) of each MFC module. As a result, the GP regression model for each MFC module is estimated based on the experimental measurements for the resistance prediction in the engineering models. In the following, we provide a brief introduction to the GP regression model, see details in [386].

$$p(\mathbf{y}|\mathbf{f}, X) \sim N(\mathbf{y}|\mathbf{m} + \mathbf{f}, \sigma^2 I) \quad (\text{F19})$$

$$p(\mathbf{f}|X) \sim N(\mathbf{f}|\mathbf{0}, \text{Cov}(X, X)) \quad (\text{F20})$$

where $X = (\mathbf{x}_1^T; \dots; \mathbf{x}_n^T)$ is a matrix of the explanatory variables, $\mathbf{y} = (y_1, \dots, y_n)^T$ is a vector of the resistivity measurement over the n samples, and σ^2 is the variance of the error. $\mathbf{m}$ is a constant mean of the resistivity governed by β_0 , and $\mathbf{f}$ follows GP with covariance,

$$\text{Cov}(\mathbf{x}_i, \mathbf{x}_j) = \sigma_f^2 \exp \left[-\frac{1}{2} \sum_{m=1}^p \frac{(x_{i,m} - x_{j,m})^2}{\sigma_m^2} \right] \quad (\text{F21})$$

where σ_f^2 controls the marginal precision of the covariance, σ_m^2 is the scaling parameter in the m -th dimension of the covariance, and p is the number of inputs. For our GP regression model, $p = 6$. After the model estimation, the GP regression models for five MFC modules are parameterized as Table F2.

Table F.2 Coefficients in GP regression models for the estimation of internal resistance

	Module 1	Module 2	Module 3	Module 4	Module 5
β_0	129.65	88.91	58.02	17.58	72.81
σ	3.11	2.19	1.34	0.80	2.32
σ_1	102.73	23.38	61.12	2.47×10^4	196.96
σ_2	0.05	1.13	0.09	0.19	0.13
σ_3	0.52	0.21	4.39×10^{12}	0.26	0.01
σ_4	2.53×10^4	12.69	14.93	3.46	59.51
σ_5	184.26	9.28×10^4	3.17×10^5	321.97	371.52
σ_6	186.48	2.67	0.04	760.30	196.20
σ_f	34.89	32.59	8.27	9.43	19.08

R_{int} can be predicted under specific experimental conditions based on Eqs. (F19)-(F20), and current generation of each MFC module can be finally obtained by the following equation:

$$I = \frac{V_{OC} - OP_{conc}}{R_{ext} + R_{int}} \quad (F22)$$

The final output of the engineering MFC model includes total current generation of five modules $I_{total} = I_{M1} + I_{M2} + I_{M3} + I_{M4} + I_{M5}$, and organic removal efficiency of the final effluent coming out of last MFC module $R_{efficiency} = \frac{S_{in} - S_{M1}}{S_{in}}$.

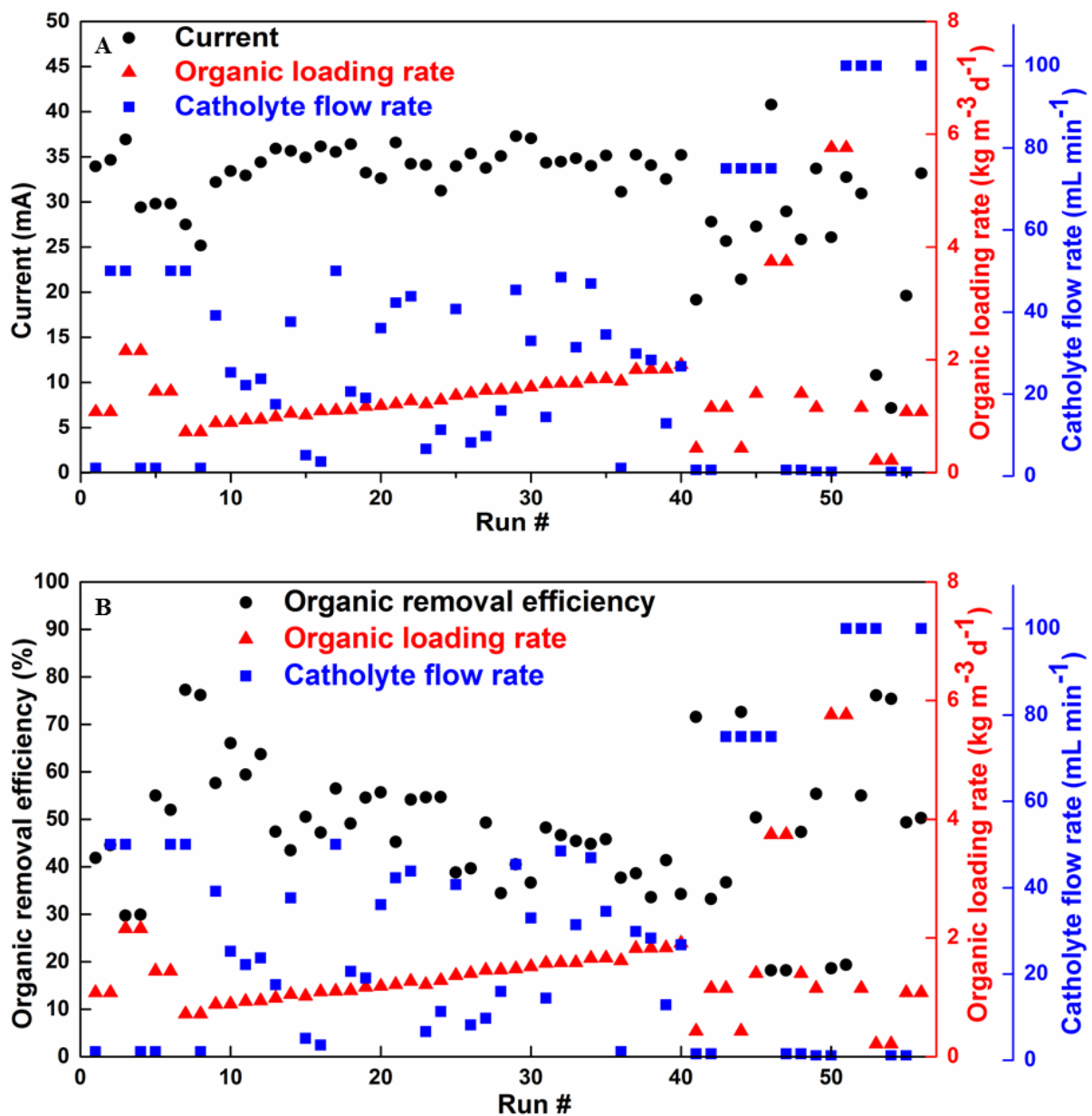


Figure F.1 Current generation and organic removal efficiency for entire five MFC modules together under all experimental runs: (A) current generation (mA); (B) organic removal efficiency (%). Note: data point shown (e.g., current generation and organic removal efficiency) is the average data at the 1st hour and the 3rd hour.

Covariance matrix for GP model and Monte Carlo Markov Chain (MCMC) evaluation

Recall that $\mathbf{x}_i = (x_{1,i}, \dots, x_{p_1,i})^T$ represents the input factors and $\mathbf{t}_i = (t_{1,i}, \dots, t_{p_2,i})^T$ represents the calibration parameters in the i -th sample. For the k -th output in $\boldsymbol{\eta}(\cdot, \cdot)$, the covariance has the form [378],

$$\text{Cov}_k((\mathbf{x}_i, \mathbf{t}_i), (\mathbf{x}_j, \mathbf{t}_j)) = \frac{1}{\lambda_{\eta,k}} e^{-d((\mathbf{x}_i, \mathbf{t}_i), (\mathbf{x}_j, \mathbf{t}_j), \boldsymbol{\beta}_{\eta,k})} \quad (\text{F23})$$

$$d((\mathbf{x}_i, \mathbf{t}_i), (\mathbf{x}_j, \mathbf{t}_j), \boldsymbol{\beta}_{\eta,k}) = \prod_{s=1}^{p_1} \beta_{\eta,s,k} (x_{i,s} - x_{j,s})^2 \prod_{s=1+p_1}^{p_1+p_2} \beta_{\eta,s-p_1,k} (t_{i,s-p_1} - t_{j,s-p_1})^2$$

The precision parameter $\lambda_{\eta,k}$ controls the marginal precision, and the $\boldsymbol{\beta}_{\eta,k} = (\beta_{\eta,1,k}, \dots, \beta_{\eta,p_1+p_2,k})^T$ vector controls the dependency strength in each dimension of $\mathbf{x}_i$ and $\mathbf{t}_i$. The smaller the $\lambda_{\eta,k}$ is, the larger the elements in the covariance matrix will be. The larger an element in $\boldsymbol{\beta}_{\eta,k}$ is, the more important the dimension corresponds to this element to control the overall covariance. As a result, the k -th output in $\boldsymbol{\eta}(\cdot, \cdot)$ follows $GP(\mathbf{m}_k, \Sigma_k^\eta)$, where $\mathbf{m}_k$ is the mean and Σ_k^η is the covariance matrix by applying Eq. (F23) to all simulation data for the k -th output. Similarly, the covariance for $\boldsymbol{\delta}(\cdot)$ at the k -th output [378],

$$\text{Cov}_k(\mathbf{x}_i, \mathbf{x}_j) = \frac{1}{\lambda_{\delta,k}} e^{-d(\mathbf{x}_i, \mathbf{x}_j, \boldsymbol{\beta}_{\delta,k})} \quad (\text{F24})$$

$d(\mathbf{x}_i, \mathbf{x}_j, \boldsymbol{\beta}_{\delta,k}) = \prod_{s=1}^{p_1} \beta_{\delta,s,k} (x_{i,s} - x_{j,s})^2$ where $\lambda_{\delta,k}$ controls the marginal precision for the discrepancy, and $\boldsymbol{\beta}_{\delta,k} = (\beta_{\delta,1,k}, \dots, \beta_{\delta,p_1,k})^T$ controls the dependency strength in each dimension of $\mathbf{x}$. As a result, the k -th output in $\boldsymbol{\delta}(\cdot)$ follows $GP(\mathbf{0}, \Sigma_k^\delta)$, where $\mathbf{0}$ is the zero mean vector and Σ_k^δ is the covariance matrix by applying Eq. (F24) to all measurement data for the k -th output. Further denote $\emptyset_j = \{\lambda_{\eta,j}, \boldsymbol{\beta}_{\eta,j}, \lambda_{\delta,j}, \boldsymbol{\beta}_{\delta,j}\}, j = 1, \dots, k$ as a set of parameters for the covariance in Eqs. (S23)-(S24).

Recall that $\mathbf{y}^M = (y_{1,1}^M; \dots; y_{1,m}^M; \dots; y_{k,1}^M; \dots; y_{k,m}^M)$ represents the output variables for all the simulation data, and $\mathbf{y}^F = (y_{1,1}^F; \dots; y_{1,n}^F; \dots; y_{k,1}^F; \dots; y_{k,n}^F)$ represents the output variables for all the measurement data. Based on Eqs. (1)-(3) and Eqs. (S23)-(S24), we have $\mathbf{y}^M \sim N(\mathbf{m}^M, \Sigma^M)$ and $\mathbf{y}^F \sim N(\mathbf{m}^F, \Sigma^F)$, $\Sigma^F = \Sigma^{\eta,F} + \Sigma^\delta + \Sigma^e$. $\mathbf{m}^M = (\mathbf{m}_1^M; \dots; \mathbf{m}_k^M)$ and $\mathbf{m}^F = (\mathbf{m}_1^F; \dots; \mathbf{m}_k^F)$ can be obtained by concatenating the mean vector $\mathbf{m}_k$ from each output of $\boldsymbol{\eta}(\cdot, \cdot)$ for the simulation data

and measurement data, respectively. $\Sigma^M = \text{diag}(\Sigma_1^{\eta,M}, \dots, \Sigma_k^{\eta,M})$ and $\Sigma^{\eta,F} = \text{diag}(\Sigma_1^{\eta,F}, \dots, \Sigma_k^{\eta,F})$ can be obtained from the covariance of each output of $\boldsymbol{\eta}(\cdot, \cdot)$ in the simulation data and measurement data, respectively. $\Sigma^\delta = \text{diag}(\Sigma_1^\delta, \dots, \Sigma_k^\delta)$ can be obtained from the covariance of each output of $\boldsymbol{\delta}(\cdot)$ in the measurement data.

For the MCMC evaluations, we performed 3000 burn-in MCMC iterations, and another 10000 MCMC iterations.

Lasso model

The input variables for the statistical model included anolyte flow rate, acetate concentration, catholyte pump rate, moisture, pH, conductivity, and their two-way interactions. A Lasso model is used as the statistical model and compared with the ensemble model,

$$\boldsymbol{\beta}(\lambda) = \min \sum_i (y_i - \mathbf{X}_i^T \boldsymbol{\beta})^2 + \lambda \|\boldsymbol{\beta}\|_1 \quad (\text{F25})$$

where $\mathbf{X}_i$ is the i -th sample of the input variables and their two-way interactions, y_i is the i -th sample of the output variable, and $\boldsymbol{\beta}$ is the model coefficient [364]. λ is a tuning parameter that controls the trade-off between the model accuracy and model complexity, which is selected with Bayesian information criterion (BIC) [364].

Residual plot analysis

The residual plots for the GP model and statistical model for current generation and organic removal efficiency under Normal_Region_90% (CV fold 1), and Extrapolation_Region were shown in Figures F3-F4. From the residual analysis plots, the model assumptions in the ensemble model and statistical model were not violated. For instance, the upper left figure in Figure F3 showed the residual plots for current generation. From the upper left and upper right subplots, the independent and identical distribution assumptions of the residual were not violated. From the bottom left and bottom right subplots, the normality assumption of the residual was not violated. Similar judgement can be obtained for other figures.

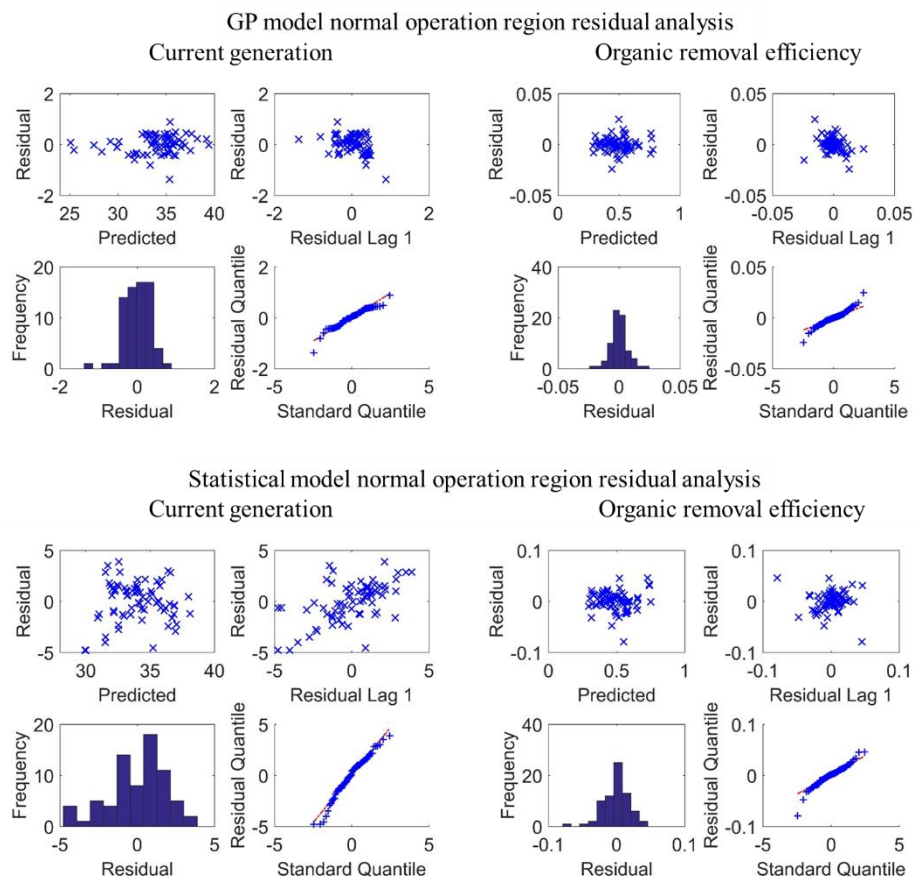
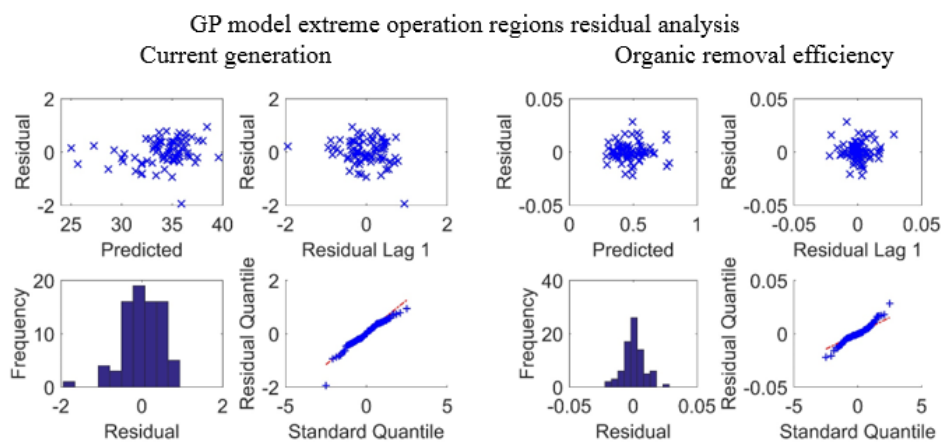


Figure F.2 Residual analysis plots for current generation and organic removal efficiency in ensemble (GP) model and statistical model under Normal_Region_90% (CV fold 1).



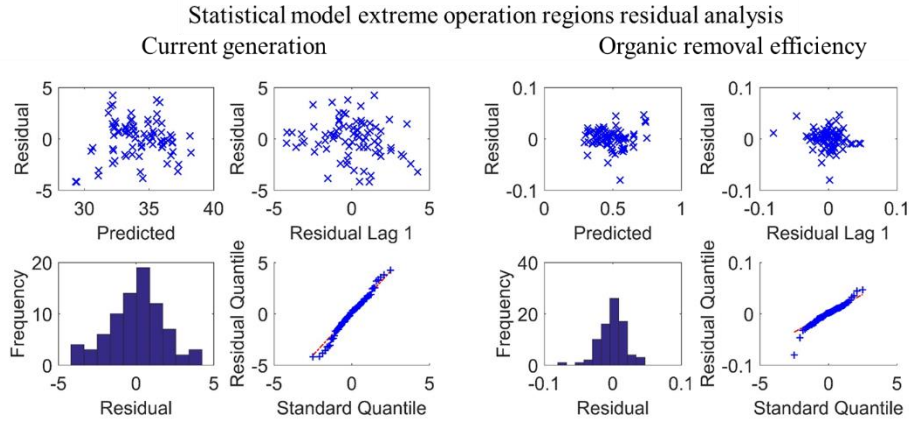


Figure F.3 Residual analysis plots for current generation and organic removal efficiency in ensemble (GP) model and statistical model under Extrapolation_Region.

Calibration parameters posterior distributions plots

The posterior distributions of the calibration parameters (Table F3) yielded from MCMC under Normal_Region_90% (CV fold 1), and Extrapolation_Region were shown in Figures F5-F6. In the figures, each diagonal plot showed the histogram of posterior samples drawn from the posterior distribution of each calibration parameter during MCMC execution. Each off diagonal plot showed the scatter plot of posterior samples drawn from the pairwise joint posterior distribution of the corresponding calibration parameters during MCMC execution. For instance, the first plot in the second row in Figure F5 showed the scatter plot of posterior samples for $q_{\max\_a}$ (horizontal axis) and $u_{\max\_a}$ (vertical axis). From the posterior distributions plots, the calibration parameters generally had a peak probability density function value, which reflected the calibrated value for the calibration parameters. Therefore, the ensemble model can help identify the calibration parameter values in the engineering models, which were represented by these peaks.

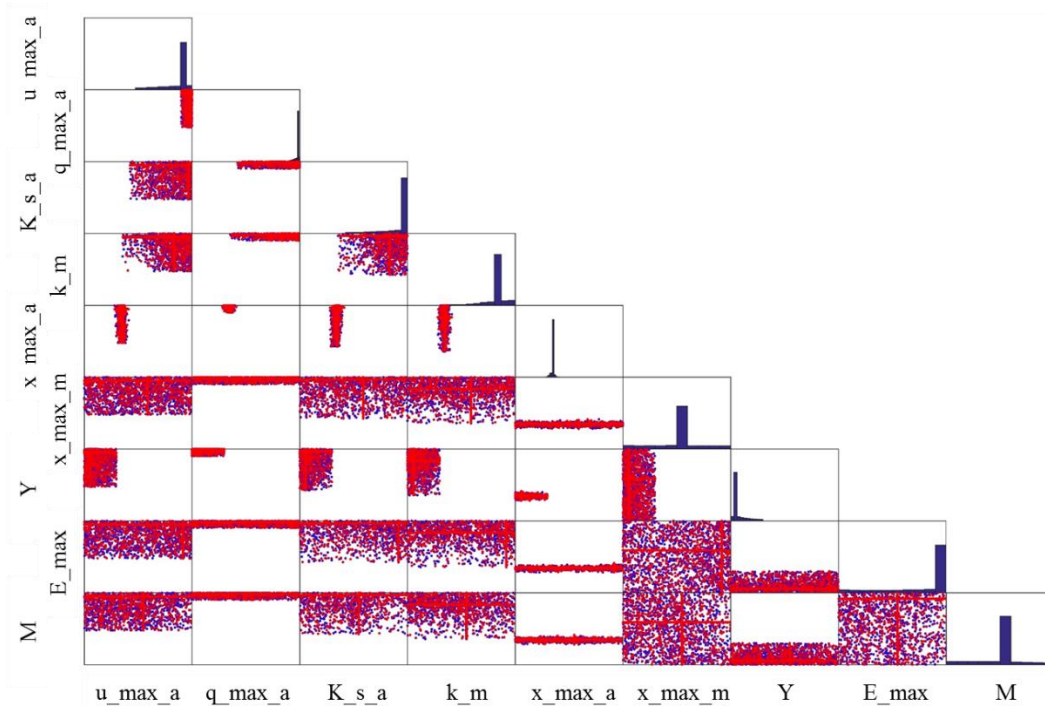


Figure F.4 Posterior distribution of calibration parameters under Normal_Region_90% (CV fold 1).

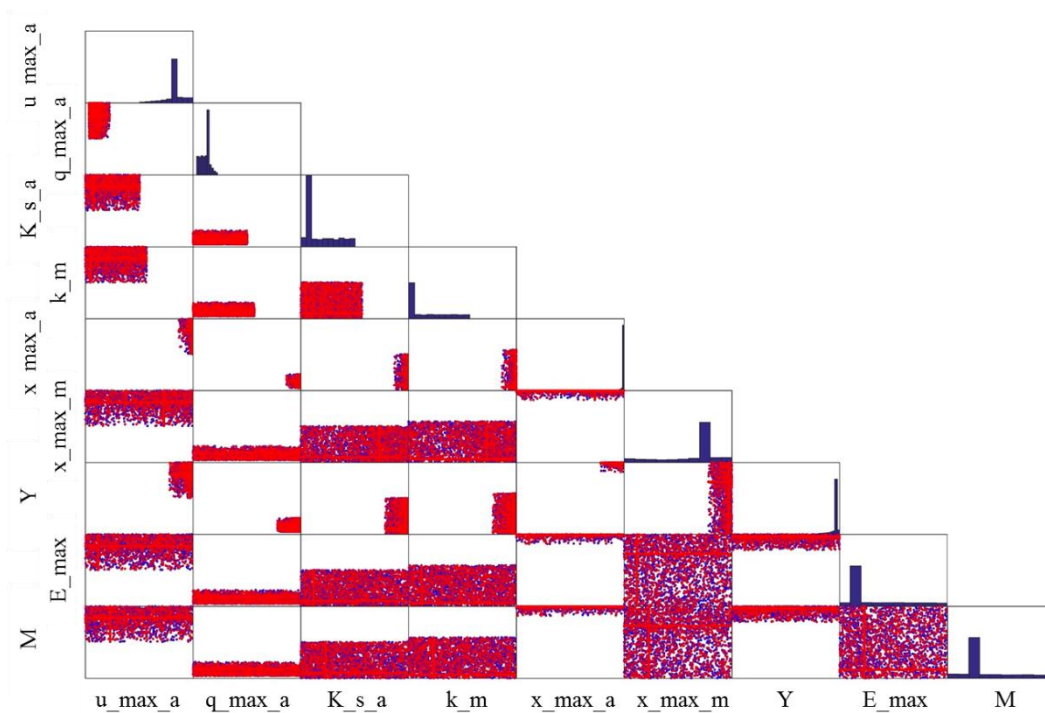


Figure F.5 Posterior distribution of calibration parameters under Extrapolation_Region.

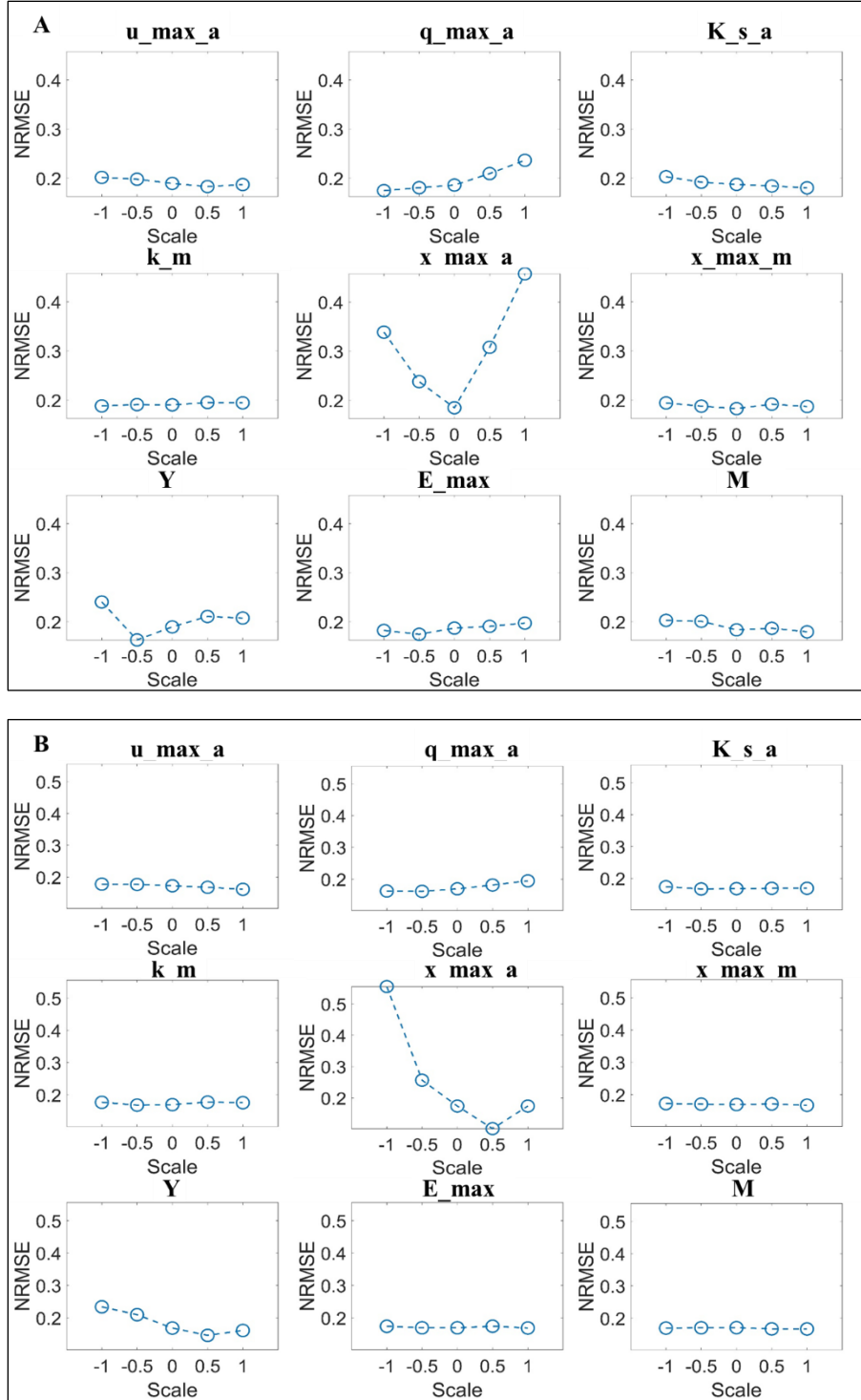


Figure F.6 Extrapolation_Region sensitivity analysis for nine calibration parameters: (A) current generation sensitivity analysis; (B) organic removal efficiency sensitivity analysis. The larger changes in NRMSE are, the more sensitive a calibration parameter is for the current output.

Appendix G for Chapter 8

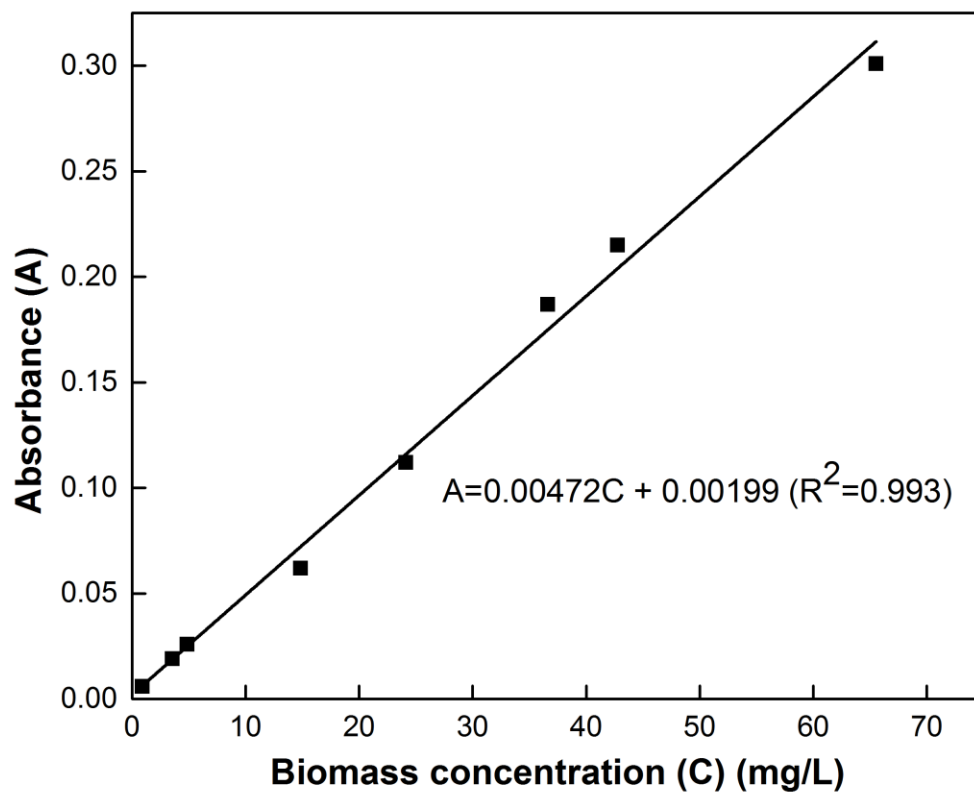


Figure G.1 The standard curve for calibration of absorbance (680 nm) against measured algal biomass concentration.

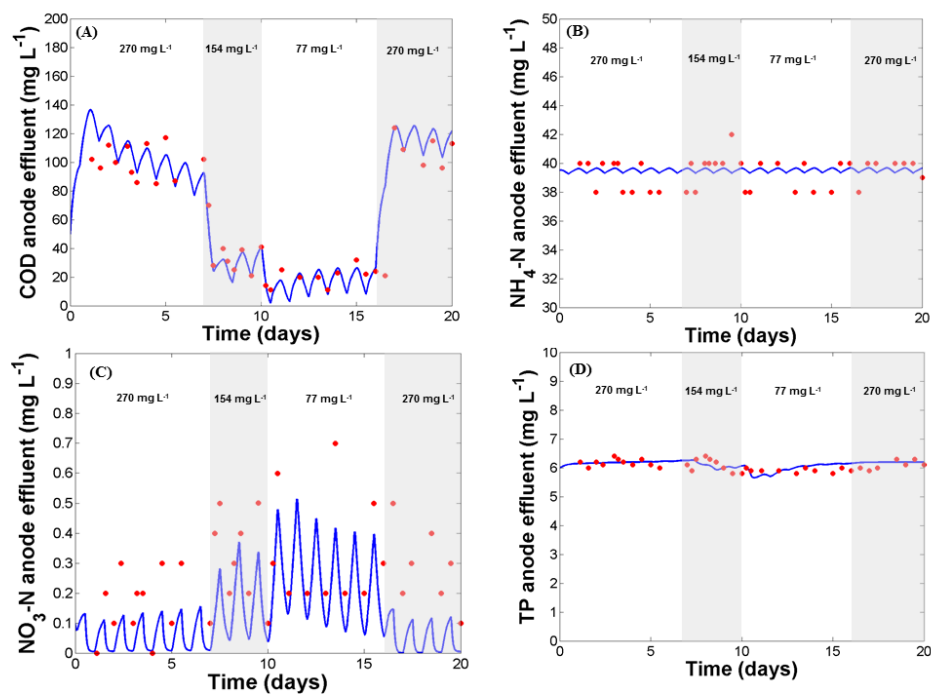
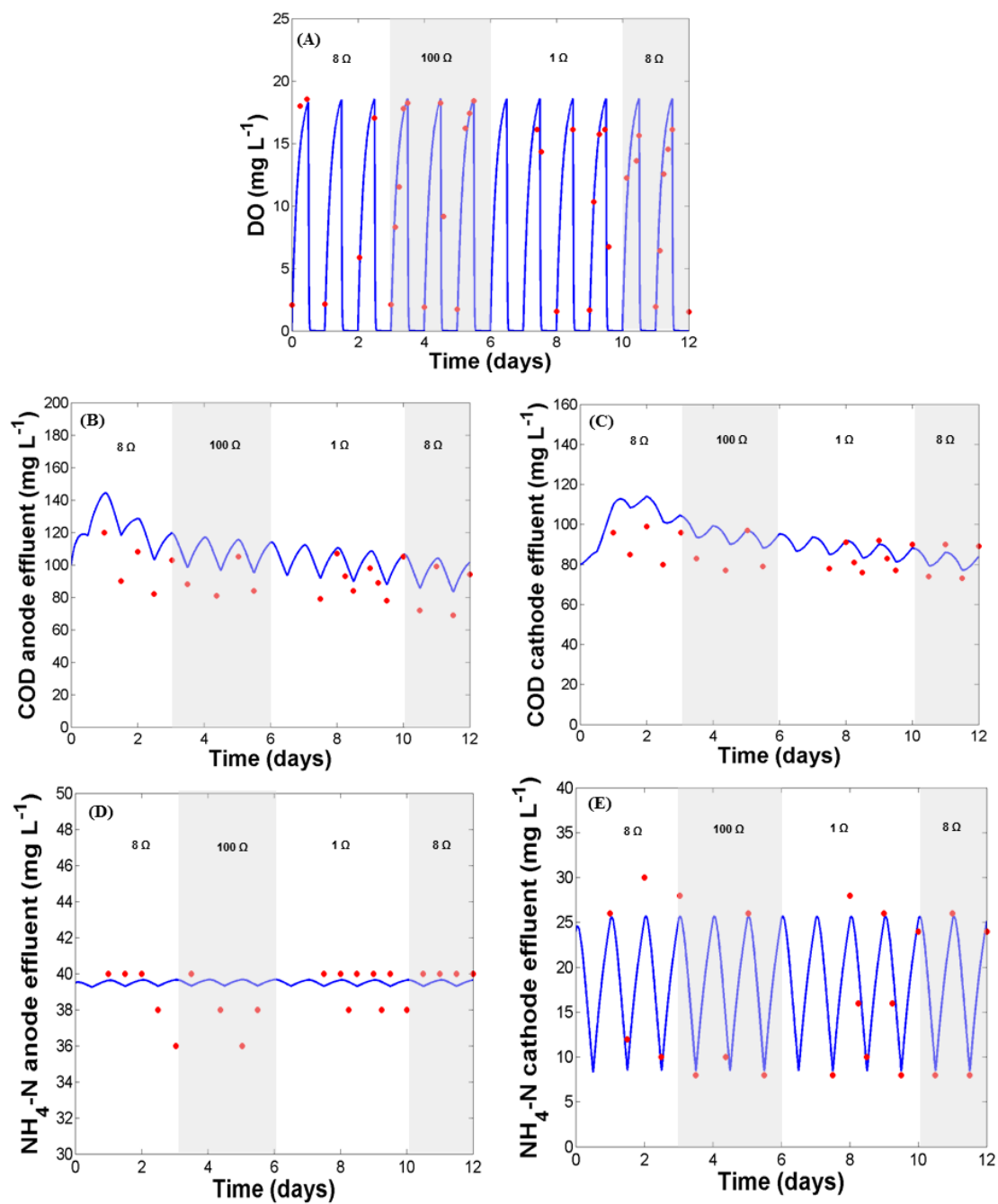


Figure G.2 Model fitting for anodic concentration of (A) COD; (B) NH₄⁺-N; (C) NO₃⁻-N; (D) TP under various influent COD concentration from 270 to 154, 77 and then back to 270 mg L⁻¹. Note: blue lines represent the simulation results; red dots represent the experimental data.



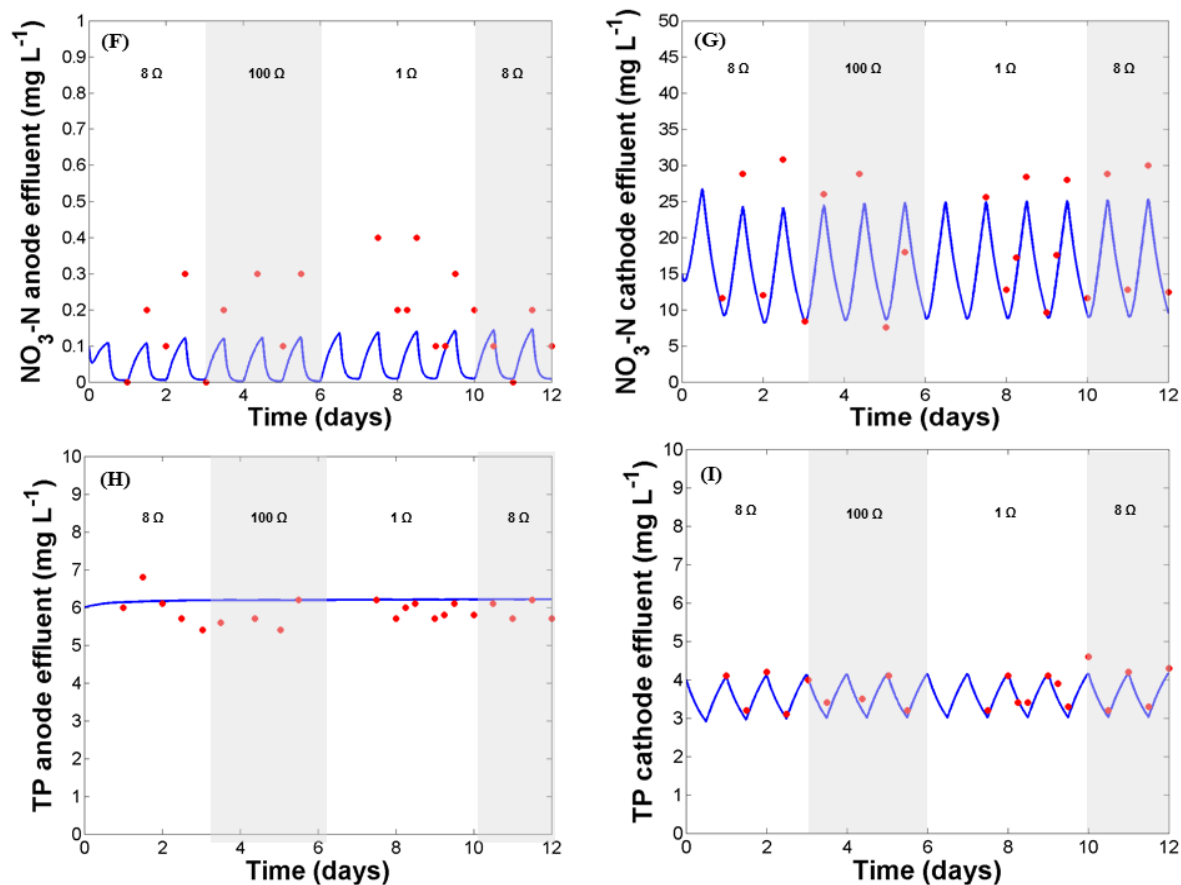
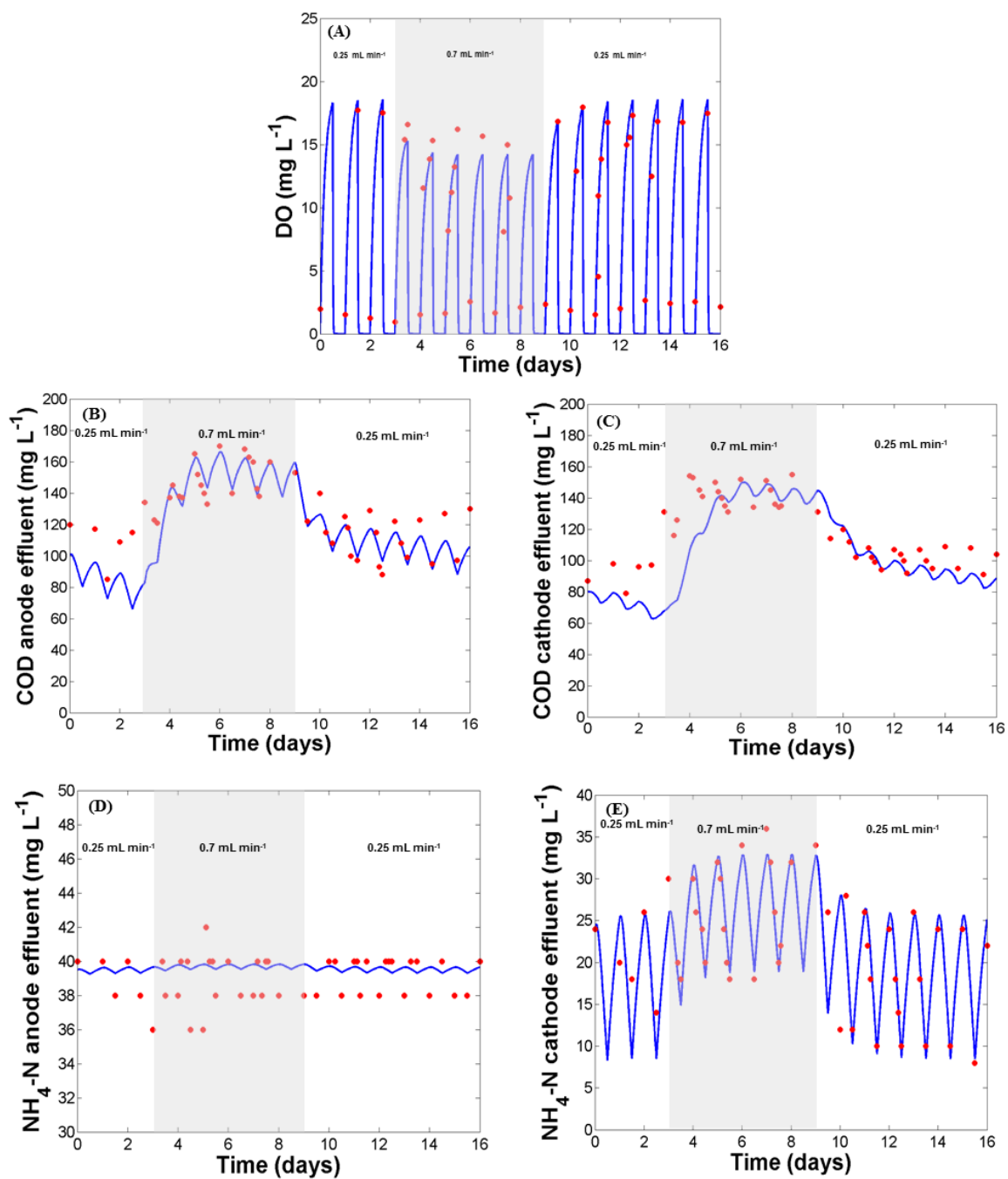


Figure G.4 Model validation with output in terms of (A) DO, (B) anode COD, (C) cathode COD, (D) anode NH₄⁺-N, (E) cathode NH₄⁺-N, (F) anode NO₃⁻-N, (G) cathode NO₃⁻-N, (h) anode TP and (I) cathode TP, when the external resistance was changed from 8 to 100, 1 and back to 8 Ω. Note: blue lines represent the simulation results; red dots represent the experimental data.



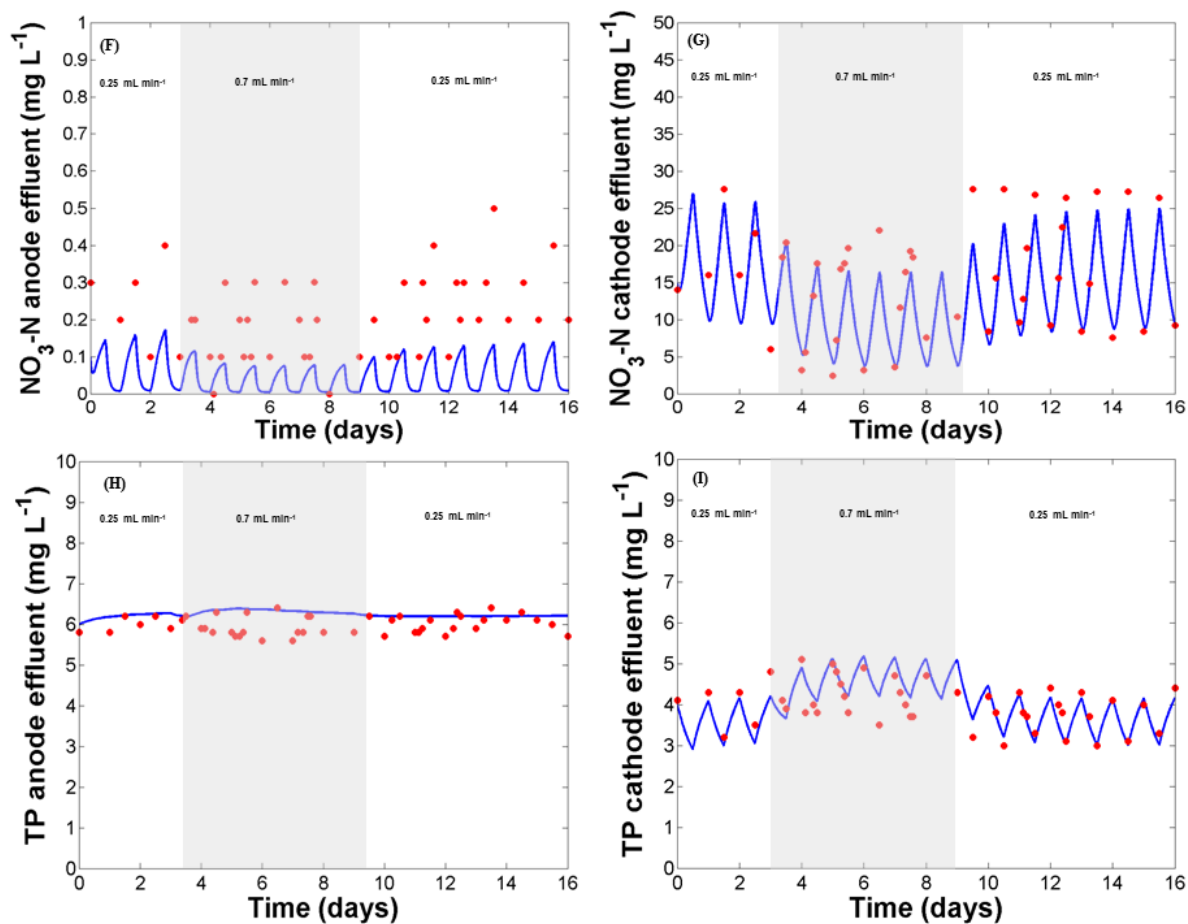
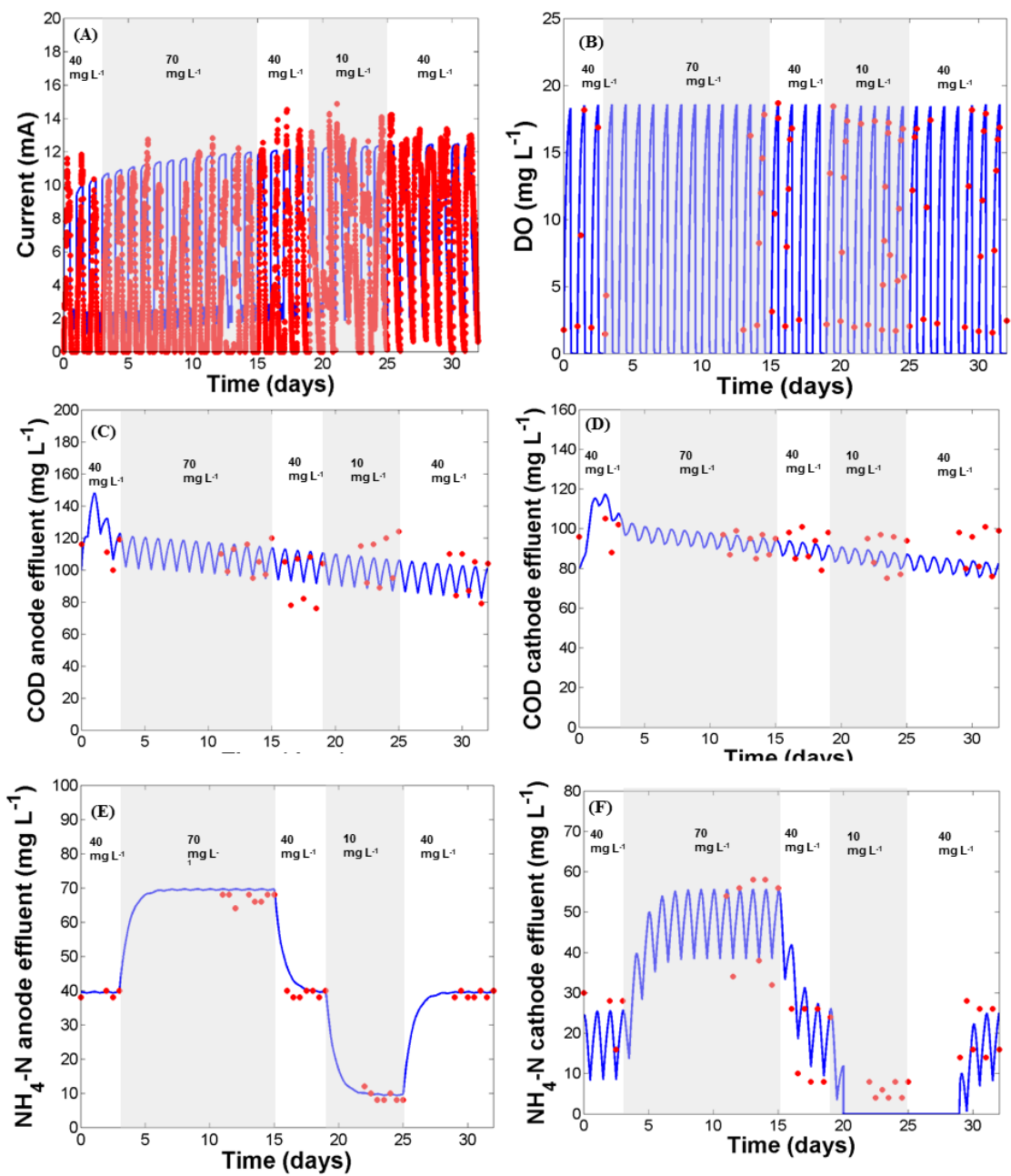


Figure G.4 Model validation with outputs in terms of (A) DO, (B) anode COD, (C) cathode COD, (D) anode $\text{NH}_4^+\text{-N}$, (E) cathode $\text{NH}_4^+\text{-N}$, (F) anode $\text{NO}_3^-\text{-N}$, (G) cathode $\text{NO}_3^-\text{-N}$, (H) anode TP and (I) cathode TP, when the flow rate was changed from 0.25 to 0.7 and back to 0.25 $\text{mL}\cdot\text{min}^{-1}$. Note: blue lines represent the simulation results; red dots represent the experimental data.



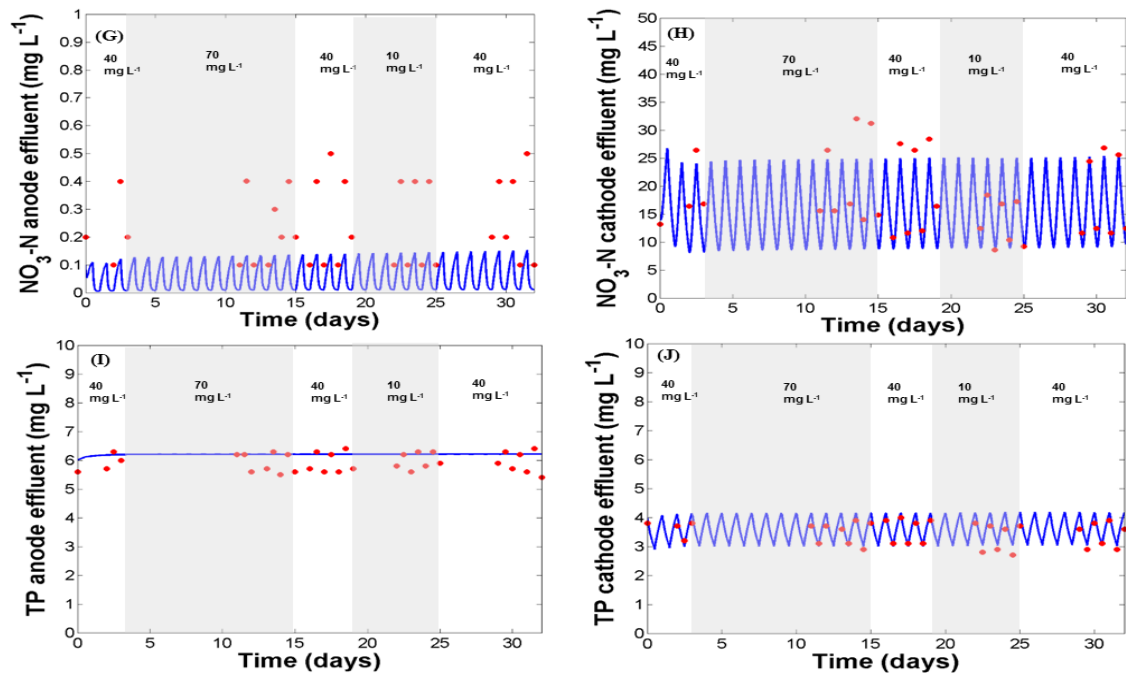
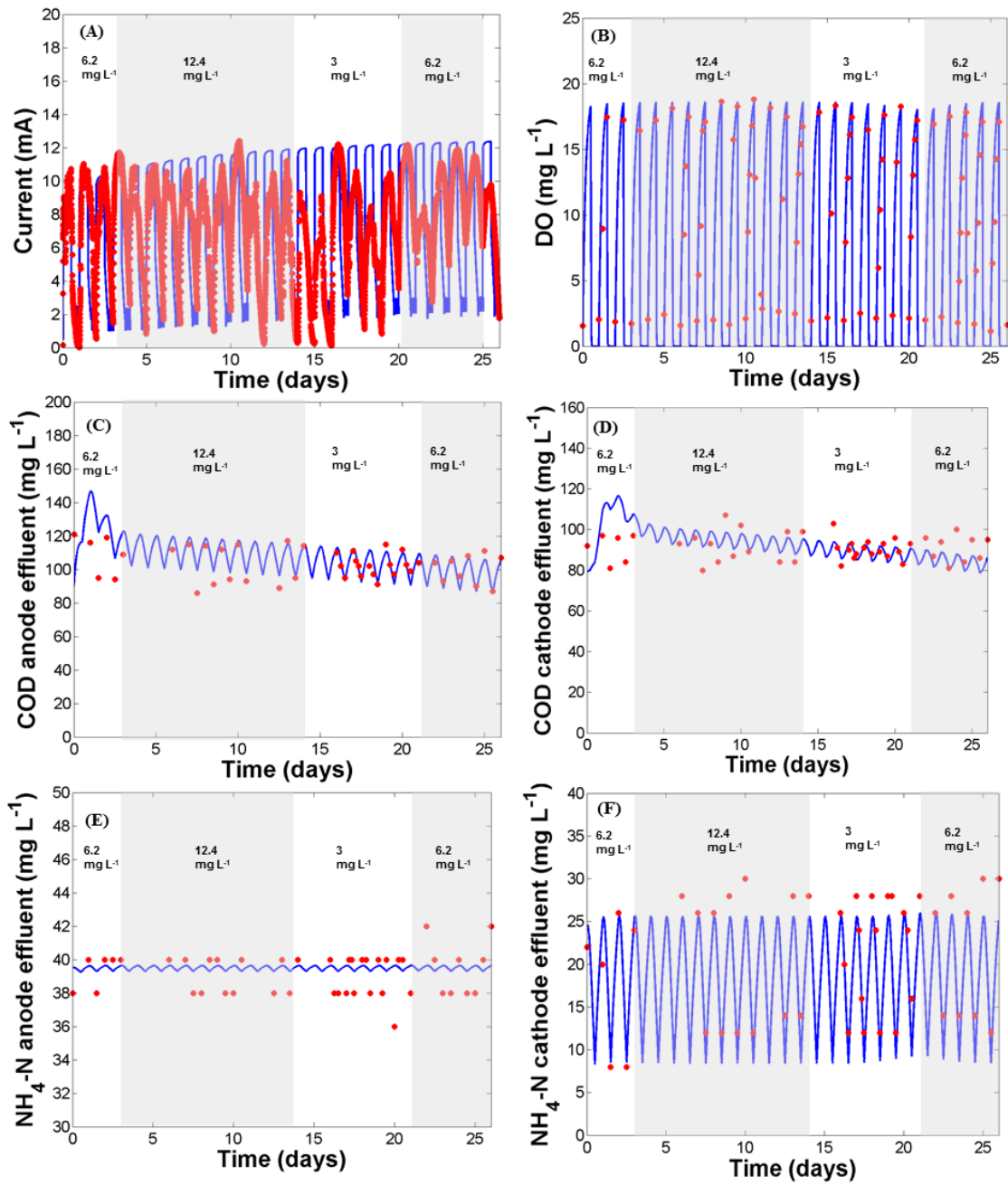


Figure G.5 Model validation with outputs in terms of (A) Current, (B) DO, (C) anode COD, (D) cathode COD, (E) anode NH₄⁺-N, (F) cathode NH₄⁺-N, (G) anode NO₃⁻-N, (H) cathode NO₃⁻-N, (I) anode TP and (J) cathode TP, when the influent NH₄⁺-N concentration was changed from 40 to 70, 40, 10 and back to 40 mg·L⁻¹. Note: blue lines represent the simulation result; red dots represent the experimental data.



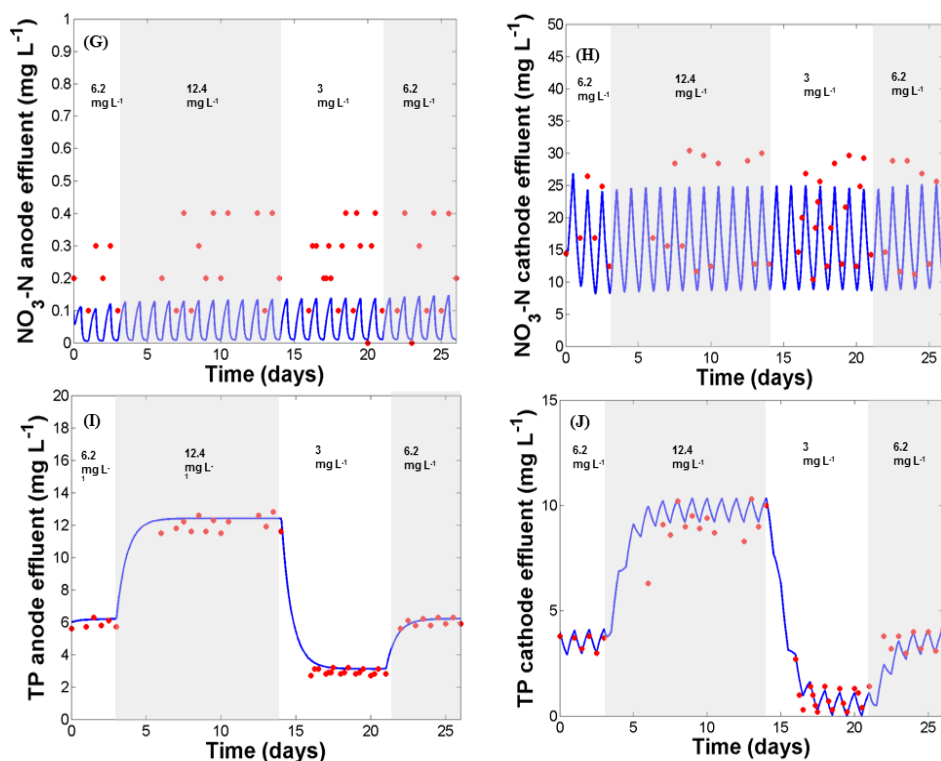


Figure G.6 Model validation with outputs in terms of (A) Current, (B) DO, (C) anode COD, (D) cathode COD, (E) anode NH₄⁺-N, (F) cathode NH₄⁺-N, (G) anode NO₃⁻-N, (H) cathode NO₃⁻-N, (I) anode TP and (J) cathode TP, when the influent TP concentration was changed from 6.2 to 12.4, 3, and back to 6.2 mg·L⁻¹. Note: blue lines represent the simulation result; red dots represent the experimental data.

Table G.4 Various Operating Conditions for IPB system

Factor Focus (Purpose)	Order	Time (day)	COD (mg L ⁻¹)	Flow rate (mL min ⁻¹)	External Resistance (Ω)	NH ₄ ⁺ -N (mg L ⁻¹)	Total phosphorus (TP) (mg L ⁻¹)
Sodium acetate (Model fitting)	1	0-7	270	0.25	8	40	6.2
	2	7-10	154	0.25	8	40	6.2
	3	10-16	77	0.25	8	40	6.2

	4	16-20	270	0.25	8	40	6.2
External Resistance (Model validation)	1	0-3	270	0.25	8	40	6.2
	2	3-6	270	0.25	100	40	6.2
	3	6-10	270	0.25	1	40	6.2
	4	10-12	270	0.25	8	40	6.2
Flow Rate (Model validation)	1	0-3	270	0.25	8	40	6.2
	2	3-9	270	0.7	8	40	6.2
	3	9-16	270	0.25	8	40	6.2
NH ₄ ⁺ -N (Model validation)	1	0-3	270	0.25	8	40	6.2
	2	3-15	270	0.25	8	70	6.2
	3	15-19	270	0.25	8	40	6.2
	4	19-25	270	0.25	8	10	6.2
	5	25-32	270	0.25	8	40	6.2
TP (Model validation)	1	0-3	270	0.25	8	40	6.2
	2	3-14	270	0.25	8	40	12.4
	3	14-21	270	0.25	8	40	3

	4	21- 26	270	0.25	8	40	6.2

Table G.2 Model assumptions.

Component	Assumptions
Anode	Well-mixing condition in anode, and mass concentration is homogeneous within anode. Thus, first-order ordinary differential equations are applied.
	Temperature and pH in anode assumed to be constant.
	Oxygen diffusion from cathode into the anode is assumed to create micro-aerobic environment, to enable nitrification without interference on anaerobic denitrification. Donnan dialysis is not considered in the ion diffusion [497, 498].
	Electrochemical reaction of NH_4^+ to NO_3^- in anode is also considered in the model.
	Some polyphosphate accumulating organisms (PAO) exist in the anode. Their uptake or release of phosphate depend on substrate supply in anode. PAO is assumed to be proportional to the anodophilic bacteria by using a constant in the model. PAO have the ability to utilize acetate along with phosphorus release, and when acetate is depleted, phosphorus can be absorbed by PAO [499-503].
	It is assumed that the transportation of every electron through external circuit can lead to migration of one molecule of NO_3^- or CH_3COO^- , or three electrons for one molecule of PO_4^{3-} to the anode to maintain charge balance in both chambers.
	Thickness of AEM is assumed to be consistent condition throughout all the experiments in this study. The impact of AEM on internal resistance is set as

	constant ($R_{membrane} = 25 \Omega$). The impact of AEM thickness on the ionic migration of various anions is also fixed, by setting the constant electromigration coefficient for various ions (e.g., α for NO_3^-).
Cathode	Well-mixing condition in cathode, and mass concentration is homogeneous within cathode. Thus, first-order ordinary differential equations are applied.
	Carbon dioxide (inorganic carbon source) is sufficient for the growth of algal biomass. Diffusion of carbon dioxide into cathode is ignored.
	The temperature and pH in the cathode is assumed to be constant. Based on the preliminary experimental data (not shown here), the cathode pH is fixed at 8.25 for the calculation of oxygen overpotential in the model.
	Because the cathode catalyst was Pt/C to ensure the Pt loading rate at 0.3 mg/cm^2 , the quality and efficiency of cathode reaction could be guaranteed. Thus, the anode and cathode overpotential caused by mass transfer and reaction rate could be neglected due to sufficient cathode reaction and buffer solution. The cathode overpotential only comes from the change of oxygen concentration.
	Light illuminance is assumed to be sufficient for algal growth, without photo-inhibition to inhibit the algal growth.
	Do not consider the inhibitory effect of over-saturated oxygen concentration on the biomass growth on cathode.
	Attached algal biomass on the carbon cloth is assumed to be constant after sufficiently long-time cultivation and operation in cathode, and the concentration is fixed for all operational mode (measured as the algal concentration at 103.4 mg L^{-1} in model simulation). Washout of attached biomass is assumed to be one

	source of suspended algal biomass, and the suspended biomass concentration measurement could indicate the growth condition of total biomass of attached and suspended biomass [504].
	Attached biomass on carbon cloth shares the same model parameters (e.g., $u_{\max}$ and k_{d-AB}) with those of the suspended algae in cathode. The attached algal biomass is also exposed to the same substrate concentration as suspended algae is in cathode.
	Only consider decayed biomass to release COD in cathode, since carbon is the major element to be released from cell lysis, and nitrogen and phosphorus release is ignored.
	Biological nitrification from NH_4^+ to NO_3^- is assumed to be instant under the condition of sufficient oxygen at daytime in the cathode, and thus the NO_2^- concentration is ignored [505].
	Biological denitrification is assumed to occur at night, due to anaerobic condition, while denitrification is ignored during daytime due to high oxygen concentration.
	It is assumed that the concentrations of nitrifying and denitrifying bacteria in the cathode are constant, and the nitrification and denitrification due to nitrifying and denitrifying bacterial activity are first-order reactions.
	Higher oxygen concentration in cathode leads to higher bacterial activity, and the oxygen consumption rate by bacterial respiration is assumed proportional to oxygen concentration in cathode.

	The impact of spatial distribution of algal biomass on the cathode for the mass transfer and nutrient removal is ignored.
	Except O ₂ , cathode reduction using other possible electron acceptors (e.g., SO ₄ ²⁻) by accepting electrons is ignored.

Description and explanations for ODEs in the main text:

The mass balance for the growth of microorganisms (anodophilic and methanogenic microorganisms), and intracellular redox mediator was established in equations (G1 to G3) [150, 166]:

$$\frac{dx_a}{dt} = \mu_a \cdot x_a - k_{da} \cdot x_a - \frac{\varepsilon_a \cdot F}{V_a} \cdot x_a \quad (\text{Eq. G1})$$

$$\frac{dx_m}{dt} = \mu_m \cdot x_m - k_{dm} \cdot x_m - \frac{\varepsilon_m \cdot F}{V_a} \cdot x_a \quad (\text{Eq. G2})$$

The assisting equations for ODEs above (Eqs. G1 and G2), and the parameter explanation are listed below, referring to previous work [166]:

$$\varepsilon_a = \frac{1 + \tanh(k_x(x_a + x_m - x_{\max-a}))}{2} \quad (\text{Eq. GA1.1})$$

$$k_{da} = 0.02 \cdot \mu_{\max-a} \quad (\text{Eq. GA1.2})$$

$$u_a = \frac{\mu_{\max-a} \cdot S_a}{k_{sa} + S_a} \cdot \frac{M_{OX}}{k_m + M_{OX}} \quad (\text{Eq. GA1.3})$$

$$\varepsilon_m = \frac{1 + \tanh(k_x(x_a + x_m - x_{\max-m}))}{2} \quad (\text{Eq. GA2.1})$$

$$k_{dm} = 0.02 \cdot \mu_{\max-m} \quad (\text{Eq. GA2.2})$$

$$u_m = \frac{\mu_{\max-m} \cdot S_a}{k_{sm} + S_a} \quad (\text{Eq. GA2.3})$$

The intracellular mediator was assumed to be generated from anodophilic bacteria as electron transferring intermediate, to aid transfer of electrons from substrate to the anode electrode based on previous finding [166]. The oxidized mediator (M_{OX}) is the mediator already releasing electrons towards the anode electrode, and then the oxidized mediator comes back to accept electrons from anodophilic bacteria to be reduced to reduced mediator (M_{RED}) to further aid electron transfer to anode electrode. Oxidized mediator (M_{OX}) is calculated in the ODE for dynamic behavior of mediator fraction (Eq. G3 and GA1.4), based on previous work [166]:

$$\frac{dM_{OX}}{dt} = -Y_M \cdot q_a + \frac{1000 \cdot 86400 \cdot \gamma \cdot I}{n_e \cdot F_a \cdot V_a \cdot x_a} \quad (\text{Eq. G3})$$

$$k_m = 0.02 \cdot m_{total} \quad (\text{Eq. G1.4})$$

where $n_e = 2$ [166]; $\gamma = 663400 \text{ mg mole}^{-1}$ [166]; $m_{total} = 0.05$ per microorganisms (dimensionless) [166]; q_a is calculated in the following equation (Eq. GA4.1), and the methanogenic substrate consumption rate is calculated in Eq. GA4.2:

$$q_a = \frac{q_{max-a} \cdot S_a}{k_{sa} + S_a} \cdot \frac{M_{OX}}{k_m + M_{OX}} \quad (\text{Eq. GA4.1})$$

$$q_m = \frac{q_{max-m} \cdot S_a}{k_{sm} + S_a} \quad (\text{Eq. GA4.2})$$

The dynamic behavior of TP in anode is described in Eq. S4:

$$\frac{dC_{P-a}}{dt} = \frac{F}{V_a} (C_{P-in} - C_{P-a}) + \frac{1000 \cdot \beta \cdot I \cdot A}{86400 \cdot F_a \cdot V_a} - p \cdot \frac{dx_a}{dt} \cdot (S_a - S_{P-lim}) \quad (\text{Eq. G4})$$

where p is a constant determining the ratio of PAO growth to the growth of anodophilic microorganisms (L mg^{-1}) based on the assumption that PAO is proportional to anodophilic bacteria to indicate the PAO growth because PAO has similarity with anodophilic bacteria to grow up better with increasing COD concentration without exceeding the upper limitation; and because the biomass fraction of PAO is relatively much smaller than that of the heterotrophs, p is set at 0.001

here [506]. PAOs have ability to uptake acetate to intrinsic biomass, and when acetate in anode is depleted, phosphorus is preferred to be taken up by PAOs [499-503]. S_{p-lim} is the determinant COD value (mg L^{-1}) above which phosphorus is released, and vice versa when COD is below the value.

The dynamic behavior of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and TP in cathode is described in Eqs. G5 to G7:

$$\frac{dC_{\text{NH}_4\text{-N-c}}}{dt} = \frac{F}{V_c} \cdot (C_{\text{NH}_4\text{-N-a}} - C_{\text{NH}_4\text{-N-c}}) - q_{\text{NH}_4\text{-N-d}} \cdot (C_{\text{AB-S}} + C_{\text{AB-A}}) - K_{\text{NH}_4\text{-N-c}} \cdot DO \quad (\text{Eq. G5.1})$$

G5.1) (for daytime)

$$\frac{dC_{\text{NH}_4\text{-N-c}}}{dt} = \frac{F}{V_c} \cdot (C_{\text{NH}_4\text{-N-a}} - C_{\text{NH}_4\text{-N-c}}) - q_{\text{NH}_4\text{-N-n}} \cdot (C_{\text{AB-S}} + C_{\text{AB-A}}) - K_{\text{NH}_4\text{-N-c}} \cdot DO \quad (\text{Eq. G5.2})$$

G5.2) (for night)

$$\frac{dC_{\text{NO}_3\text{-N-c}}}{dt} = \frac{F}{V_c} \cdot (C_{\text{NO}_3\text{-N-a}} - C_{\text{NO}_3\text{-N-c}}) - q_{\text{NO}_3\text{-N-d}} \cdot (C_{\text{AB-S}} + C_{\text{AB-A}}) + K_{\text{NH}_4\text{-N-c}} \cdot DO - \frac{86400 \cdot 1000 \cdot \alpha \cdot I \cdot A}{F_a \cdot V_c} \quad (\text{Eq. G6.1})$$

(for daytime)

$$\frac{dC_{\text{NO}_3\text{-N-c}}}{dt} = \frac{F}{V_c} \cdot (C_{\text{NO}_3\text{-N-a}} - C_{\text{NO}_3\text{-N-c}}) - q_{\text{NO}_3\text{-N-n}} \cdot (C_{\text{AB-S}} + C_{\text{AB-A}}) + K_{\text{NH}_4\text{-N-c}} \cdot DO -$$

$$K_{\text{NO}_3\text{-N-cn}} \cdot C_{\text{NO}_3\text{-N-c}} \cdot S_c - \frac{86400 \cdot 1000 \cdot \alpha \cdot I \cdot A}{F_a \cdot V_c} \quad (\text{Eq. G6.2})$$

(for night)

$$\frac{dC_{\text{P-c}}}{dt} = \frac{F}{V_c} \cdot (C_{\text{P-a}} - C_{\text{P-c}}) - q_{\text{P-d}} \cdot (C_{\text{AB-S}} + C_{\text{AB-A}}) - \frac{86400 \cdot 1000 \cdot \beta \cdot I \cdot A}{F_a \cdot V_c} \quad (\text{Eq. G7.1})$$

(for daytime)

$$\frac{dC_{\text{P-c}}}{dt} = \frac{F}{V_c} \cdot (C_{\text{P-a}} - C_{\text{P-c}}) - q_{\text{P-n}} \cdot (C_{\text{AB-S}} + C_{\text{AB-A}}) - \frac{86400 \cdot 1000 \cdot \beta \cdot I \cdot A}{F_a \cdot V_c} \quad (\text{Eq. G7.2})$$

(for night)

The basic equation to calculate current generation considering overpotential and internal resistance is listed below, based on previous work [166], and extra potential caused by oxygen concentration is also included:

$$I \cdot R_{ext} = V_{OC} - OP_{anode} - OP_{cathode} - OP_{conc} - OP_{oxygen} - I \cdot R_{int} \quad (\text{Eq. G8})$$

The equations to assist calculation of Eq. S8 above and Eq. 8 in the main text are listed below, with the assumption that anode potential can be ignored, and cathode overpotential only comes from oxygen based on our findings in previous work [166]:

$$V_{OC} = V_{min} + (V_{max} - V_{min}) \cdot e^{-\frac{1}{k_r \cdot x_a}} \quad (\text{Eq. GA5.1})$$

$$R_{int} = R_{min} + (R_{max} - R_{min}) \cdot e^{-k_r \cdot x_a} + R_{membrane} \quad (\text{Eq. GA5.2})$$

$$OP_{conc} = \frac{R \cdot T}{F_a} \ln\left(\frac{M_{total}}{M_{red}}\right) \quad (\text{Eq. GA5.3})$$

$$m_{total} = M_{red} + M_{ox} \quad (\text{Eq. GA5.4})$$

Root mean square error (RMSE) [161]

The RMSE calculation is to obtain the relative error from simulation result to true value by calculating the standard deviation based on true value in numerator, divided by the maximum value of the experimental data to normalize the error in the denominator.

$$RMSE = \frac{\sqrt{\frac{\sum_{i=1}^N (y_i - \hat{y}_i)^2}{N}}}{\max(\hat{y})} \quad (\text{Eq. G9})$$

Sensitivity analysis [166]

The strategy of sensitivity analysis refers to previous studies [166]. The method to determine parameter sensitivity is to only change one parameter at one time while other parameters are fixed at their original values, and obtain the response of model output based on the following equations:

$$s = \left| \frac{p_i^o(R_1 - R^o)}{R^o(10p_i^o - p_i^o)} \right| + \left| \frac{p_i^o(R_2 - R^o)}{R^o(0.1p_i^o - p_i^o)} \right| \quad (\text{Eq. G10})$$

where p_i^o is the original value of targeted parameter; R^o is the original model output (multiplication of current generation (mA) and suspended algal biomass concentration (mg L^{-1}) ($R^o = I * C_{AB-S}$)); R_1 and R_2 are new model outputs of increasing and decreasing the targeted parameter by one magnitude respectively; s is the sum of two absolute value derived from the variation of output value due to single parameter change. The sensitivity of each parameter is normalized based on the greatest value and ranked, and the results for top 10 parameters are shown in Table G3, showing the parameters with priority to be adjusted in the model.

Table G.3 Sensitivity analysis ranked top 10 parameters (among all 55 parameters) and parameters related to ionic migration through AEM.

Rank	Parameter	Relative Sensitivity Measure
1	Maximum specific growth rate of anodophilic microorganisms (μ_{max-a})	1
2	Maximum specific growth rate of algal biomass in cathode (μ_{max-AB})	0.857
3	Highest circuit voltage (V_{max})	0.819
4	Weighing factor for TP in cathode (ϕ_P)	0.758
5	Half-rate constant for anodophilic microorganisms (k_{sa})	0.721
6	Nitrification constant in anode for converting $\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$ ($K_{\text{NH}_4\text{-N-a}}$)	0.566
7	Weighing factor for COD in cathode (ϕ_{COD})	0.511
8	Constant determining the speed of internal resistance responding to the change in microorganism concentration (k_r)	0.494

9	Fraction constant of diffused oxygen from cathode to anode (D_d)	0.469
10	Photosynthesis constant (D_p)	0.433
25	Electromigration coefficient of PO_4^{3-} from cathode to the anode (β)	0.318
31	Electromigration coefficient of acetate ion (CH_3COO^-) from cathode to the anode (θ)	0.302
42	Electromigration coefficient of NO_3^- from cathode to the anode (α)	0.267

Table G.4 Symbol and constants.

	Definition	Value (shown if as model parameter)	Equations where symbols appear Number
S_a	COD concentration in anode (mg L^{-1})	Output	(8.1) (8.3) (G4) (8.6.1) (8.6.2) (GA1.3) (GA2.3) (GA4.1) (GA4.2)
S_c	COD concentration in cathode (mg L^{-1})	Output	(8.4) (8.6.1) (8.6.2) (G6.1) (G6.2)
S_{in}	COD concentration in the influent (mg L^{-1})	Input	(8.1)
$C_{\text{NH}_4\text{-N-in}}$	Influent NH_4^+ -N concentration (mg L^{-1})	Input	(8.2)
$C_{\text{NH}_4\text{-N-a}}$	NH_4^+ -N concentration in the anode effluent (mg L^{-1})	Output	(8.2) (G5.1) (G5.2)
$C_{\text{NH}_4\text{-N-c}}$	NH_4^+ -N concentration in the cathode effluent (mg L^{-1})	Output	(8.4) (G5.1) (G5.2)
$C_{\text{NO}_3\text{-N-in}}$	Nitrate concentration in the anode influent (mg L^{-1})	0 (constant)	(8.3)
$C_{\text{NO}_3\text{-N-a}}$	Nitrate concentration in the anode effluent (mg L^{-1})	Output	(8.1) (8.3) (G6.1) (G6.2)
$C_{\text{NO}_3\text{-N-c}}$	Nitrate concentration in the cathode effluent (mg L^{-1})	Output	(8.4) (8.6.1) (8.6.2) (G6.1) (G6.2)
C_{P-in}	Phosphorus concentration in the anode influent (mg L^{-1})	Input	(G4)
C_{P-a}	Phosphorus concentration in the anode effluent (mg L^{-1})	Output	(G4) (G7.1) (G7.2)

C_{P-c}	Phosphorus concentration in the cathode effluent (mg L ⁻¹)	Output	(8.4) (G7.1) (G7.2)
C_{AB-S}	Concentration of suspended biomass in cathode (mg L ⁻¹)	Output	(8.4) (G5.1) (8.6.1) (8.6.2) (G5.1) (G5.2) (G6.1) (G6.2) (G7.1) (G7.2)
C_{AB-A}	Concentration of attached biomass in cathode (mg L ⁻¹)	103.4 (constant)	(8.4) (8.5.1) (8.6.1) (8.6.2) (G5.1) (G5.2) (G6.1) (G6.2) (G7.1) (G7.2)
DO	Dissolved oxygen concentration in the cathode (mg L ⁻¹);	Output	(8.1) (8.2) (8.3) (8.5.1) (8.5.2) (6.1) (8.6.2) (G5.1) (G5.2) (G6.1) (G6.2)
x_a	Concentration of anodophilic bacteria in anode (mg L ⁻¹)	Output	(G4) (G1) (G2) (G3) (GA1.1) (GA2.1) (GA5.1) (GA5.2)
x_m	Concentration of methanogenic bacteria in anode (mg L ⁻¹)	Output	(G2) (GA1.1) (GA2.1)
I	Current (A)	Output	(8.2) (8.3) (G4) (G3) (8.5.1) (8.5.2) (8.6.1) (8.6.2) (G6.1) (G6.2) (G7.1) (G7.2) (8.7) (8.8)
V_a	Anode volume (L)	0.5 (constant)	(8.1) (8.2) (8.3) (G4) (G1) (G2) (G3)
V_c	Cathode volume (L)	0.45 (constant)	(8.4) (8.5.1) (8.5.2) (8.6.1) (8.6.2) (G5.1) (G5.2) (G6.1) (S6.2) (G7.1) (G7.2)
F	Flow rate (L day ⁻¹)	Input	(8.1) (8.2) (8.3) (G4) (8.5.1) (8.5.2) (8.6.1) (8.6.2) (G5.1) (G5.2) (G6.1) (G6.2) (G7.1) (G7.2) (G1) (G2)
R_{ext}	External resistance (Ω)	Input	(8.8) (G8)
R_{int}	Internal resistance (Ω)	Intermediate output	(8.8) (G8)
R_{min}	Minimum internal resistance (Ω)	15	(GA5.2)
R_{max}	Maximum internal resistance (Ω)	2000	(GA5.2)
$R_{membrane}$	Membrane resistance (Ω)	25	(GA5.2)
V_{OC}	Open circuit voltage (V)	Intermediate output	(8.8) (G8) (GA5.1)
V_{min}	Lowest circuit voltage (V)	0.1	(GA5.1)

V_{max}	Highest circuit voltage (V)	0.78	(GA5.1)
OP_{anode}	Anode overpotential	Assumed to be 0	(G8)
$OP_{cathode}$	Cathode overpotential	Assumed to be 0	(G8) (GA5.3)
OP_{conc}	Concentration overpotential	Intermediate output	(8.8) (G8)
OP_{oxygen}	Oxygen overpotential	Intermediate output	(8.7) (8.8) (G8)
A	AEM area (dm ²)	4 (constant)	(8.) (8.3) (G4) (8.6.1) (8.6.2) (G6.1) (G6.2) (G7.1) (G7.2)
R	Ideal gas constant (J K ⁻¹ mol ⁻¹)	8.314 (constant)	(8.7) (GA5.3)
T	Temperature (K)	298.15 (constant)	(8.7) (GA5.3)
F_a	Faraday constant (C mol ⁻¹) or (A s mol ⁻¹)	96485 (constant)	(8.1) (8.2) (8.3) (G4) (8.5.1) (5.2) (8.6.1) (8.6.2) (8.6.1) (8.6.2) (G7.1) (G7.2) (8.7) (G3) (GA5.3)
x_{max-a}	Maximum attainable concentrations for anodophilic bacteria (mg L ⁻¹)	80	(GA1.1)
x_{max-m}	Maximum attainable concentrations for methanogenic bacteria (mg L ⁻¹)	550	(GA2.1)
μ_a	Specific growth rate of anodophilic microorganisms (day ⁻¹)	Intermediate output	(G1) (GA1.3)
μ_m	Specific growth rate of methanogenic microorganisms (day ⁻¹)	Intermediate output	(G2) (GA2.3)
μ_{max-a}	Maximum specific growth rate of anodophilic microorganisms (day ⁻¹)	0.95	(GA1.2) (GA1.3)
μ_{max-m}	Maximum specific growth rate of methanogenic microorganisms (day ⁻¹)	0.4	(GA2.2) (GA2.3)
q_a	COD consumption rate by anodophilic bacteria (mg day ⁻¹)	Intermediate output	(8.1) (G3) (GA4.1)
q_m	COD consumption rate by methanogenic bacteria (day ⁻¹)	Intermediate output	(8.1) (GA4.2)
q_{max-a}	Maximum anodophilic COD reaction rate (day ⁻¹)	0.3	(GA4.1)
q_{max-m}	Maximum methanogenic COD reaction rate (day ⁻¹)	0.3	(GA4.2)

q_{S-d}	Carbon uptake constant by biomass in cathode at daytime (day^{-1})	0.135	(8.6.1)
q_{S-n}	Carbon uptake constant by biomass in cathode at night (day^{-1})	0.015	(8.6.2)
$q_{\text{NH}_4^+-N-d}$	NH_4^+ -N uptake constant by biomass in cathode at daytime (day^{-1})	0.0015	(G5.1)
$q_{\text{NH}_4^+-N-n}$	NH_4^+ -N uptake constant by biomass in cathode at night (day^{-1})	0.015	(G5.2)
$q_{\text{NO}_3^--N-d}$	NO_3^- -N uptake constant by biomass in cathode at daytime (day^{-1})	0.006	(G6.1)
$q_{\text{NO}_3^--N-n}$	NO_3^- -N uptake constant by biomass in cathode at night (day^{-1})	0.004	(G6.2)
q_{P-d}	Phosphorus uptake constant by biomass in cathode at daytime (day^{-1})	0.0225	(G7.1)
q_{P-n}	Phosphorus uptake constant by biomass in cathode at night (day^{-1})	0.006	(G7.2)
k_r	Constant determining the speed of internal resistance responding to the change in microorganism concentration (L mg^{-1})	0.12	(GA5.1) (GA5.2)
k_x	Steepness factors for anodophilic and methanogenic microorganism (L mg^{-1})	0.01	(GA1.1) (GA2.1)
k_{sa}	Half-rate constant for anodophilic microorganisms (mg L^{-1})	15	(GA1.3) (GA4.1)
k_{sm}	Half-rate constant for methanogenic microorganisms (mg L^{-1})	80	(GA2.3) (GA4.2)
k_m	Half-rate constant of mediator (dimensionless)	0.01	(GA1.3) (GA1.4) (GA4.1)
k_{da}	Decay rate of anodophilic microorganism (day^{-1})	0.19	(G1) (GA1.2)
k_{dm}	Decay rate of methanogenic microorganism (day^{-1})	0.08	(G2) (GA2.2)
k_{d-AB}	Decay rate of algal biomass (day^{-1})	0.05	(8.6.1) (8.6.2)
d_c	Conversion constant from decayed algal biomass to COD in cathode (dimensionless)	0.8	(8.6.1) (8.6.2)
ε_a	Biofilm retention constant for anodophilic bacteria (dimensionless)	Intermediate output	(G1) (GA1.1)

ε_m	Biofilm retention constant for methanogenic bacteria (dimensionless)	Intermediate output	(G2) (GA2.1)
M_{OX}	Fraction of oxidized mediator versus total mediator fraction per microorganisms (dimensionless)	Intermediate output	(G3) (GA1.3) (GA4.1) (GA5.4)
M_{red}	Fraction of reduced mediator versus total mediator fraction per microorganisms (dimensionless)	Intermediate output	(GA5.3) (GA5.4)
M_{total}	Total mediator fraction per microorganisms (dimensionless)	0.05 (constant)	(GA1.4) (GA5.3) (GA5.4)
Y_M	Mediator yield (dimensionless)	20	(G3)
γ	Mediator molar mass (mg mole ⁻¹)	663400 (constant)	(G3)
n_e	Number of electrons transferred per mole of mediator	2 (constant)	(G3)
K_{NH_4-N-a}	Constant for nitrification in anode for converting NH ₄ ⁺ -N to NO ₃ ⁻ -N (day ⁻¹)	0.0005	(8.2) (8.3)
K_{NH_4-N-c}	Constant for nitrification in cathode for converting NH ₄ ⁺ -N to NO ₃ ⁻ -N (day ⁻¹)	0.0025	(8.5.1) (8.5.2) (G5.1) (G5.2) (G6.1) (G6.2)
K_{NO_3-N-a}	Constant for denitrification reaction in anode (L mg ⁻¹)	0.0675	(8.1) (8.3)
K_{NO_3-N-cn}	Constant for denitrification reaction in cathode at night (L mg ⁻¹)	0.003	(8.6.2) (G6.2)
K_{resp}	Conversion coefficient to remove COD by bacterial respiration in cathode (dimensionless)	0.7	(8.6.1) (8.6.2)
K_H	Henry's constant for oxygen	769.23 (constant)	(8.7)
I_{NH_4-N-a}	Electrochemical reaction constant by using NH ₄ ⁺ -N as electron donor (g mol ⁻¹)	10	(8.2) (8.3)
M	Molar mass of oxygen (g mol ⁻¹)	32 (constant)	(8.7)
D_r	Constant for fraction of diffused oxygen to react with organic in anode (dimensionless)	0.0402	(8.1)
D_d	Fraction constant of diffused oxygen from cathode to anode (dimensionless)	0.1	(8.1) (8.3) (8.5.1) (8.5.2)
D_p	Photosynthesis constant (day ⁻¹)	370	(8.5.1)

D_{cr-n}	Reaction constant of bacterial respiration in cathode at night (day ⁻¹)	50	(8.5.2)
D_{cr-d}	Reaction constant of bacterial respiration in cathode at daytime (day ⁻¹)	2	(8.5.1)
θ	Electromigration coefficient of acetate (CH ₃ COO ⁻) from cathode to the anode (g mol ⁻¹ dm ⁻²)	0.0002	(8.1) (8.6.1) (8.6.2)
α	Electromigration coefficient of NO ₃ ⁻ from cathode to the anode (g mol ⁻¹ dm ⁻²)	0.0007	(8.3) (G6.1) (G6.2)
β	Electromigration coefficient of PO ₄ ³⁻ from cathode to the anode (g mol ⁻¹ dm ⁻²)	0.0001	(8.4) (G7.1) (G7.2)
p	Constant for ratio of PAO growth versus the growth of anodophilic microorganisms (L mg ⁻¹)	0.001	(G4)
S_{p-lim}	Upper limit of carbon source concentration at which carbon source is sufficient for PAO to uptake and release phosphorus to outside (mg L ⁻¹)	100	(G4)
μ_{max-AB}	Maximum specific growth rate of algal biomass in cathode (day ⁻¹)	1.335	(8.4)
φ_{COD}	Weighing factor for COD in cathode (dimensionless)	0.25	(8.4)
φ_{NH_4}	Weighing factor for NH ₄ ⁺ -N in cathode (dimensionless)	11	(8.4)
φ_{NO_3}	Weighing factor for NO ₃ ⁻ -N in cathode (dimensionless)	3.8	(8.4)
φ_P	Weighing factor for TP in cathode (dimensionless)	0.219	(8.4)
K_{C-AB}	Half saturation constants for carbon for biomass growth (mg L ⁻¹)	0.35	(8.4)
K_{NH_4-N-AB}	Half saturation constants for NH ₄ ⁺ -N for biomass growth (mg L ⁻¹)	0.0048	(8.4)
K_{NO_3-N-AB}	Half saturation constants for NO ₃ ⁻ -N for biomass growth (mg L ⁻¹)	0.2	(8.4)
K_{P-AB}	Half saturation constants for TP for biomass growth (mg L ⁻¹)	0.02	(8.4)

Appendix H for Chapter 9

Table H.1 Experimental design for testing different MMs ability of algal attachment (an initial algal concentration at 1 g L⁻¹)

Total operational cycles (operational period)	Experimental group #	Material # 1 (pore size mm)	Material # 2 (pore size mm)
4 (16 days)	1	Polyester (0.11)	Nylon (0.11)
	2	Polyester (0.53)	Nylon (0.53)
	3	Polyester (4.98)	Nylon (5.31)
8 (32 days)	4	Polyester (0.53)	Polyester (0.11)

Table H.2 Experimental design for the IPB system equipped with MM.

Group #	Number of MM (size of each MM)	Time interval (days) for algal harvesting	Catholyte mixing	Total operation period (days) (cycle number)
1	1 (392 cm ²)	6	Stirring	18 (3)
	1 (392 cm ²)	4	Stirring	12 (3)
	1 (392 cm ²)	8	Stirring	24 (3)
2	3 (130.7 cm ²)	2 for each MM in order	Stirring	18 (3)
	2 (196 cm ²)	3 for each MM in order	Stirring	18 (3)
	1 (392 cm ²)	6	Stirring	18 (3)
3	N/A	N/A	Aeration	19
	3 (130.7 cm ²)	2 for each MM in order	Stirring	48 (8)
	3 (130.7 cm ²)	2 for each MM in order	Recirculation	48 (8)

Table H.3 Algal productivity comparison with other studies of the IPB system with tubular MFCs

Algal inocula	Harvesting method [#]	Algal productivity (g m ⁻³ (volume of treated wastewater))*	Reference
Mixed algal inocula from wild pond	Catholyte filtration	70.4	[424]
<i>Pseudokirchneriella subcapitata</i>	Manually scraped	12.6	[3]
Mixed algal inocula from wastewater treatment facilities	/	47.8	[4]
Mixed algal inocula from wild pond	Manually scraped	72.3	This study

*Note: the algal productivities of all studies are calculated normalized to the treated wastewater in the algal reactor (cathode)

#Note: the symbol ‘/’ indicated that there was no algal harvesting in the study, and the algal concentration was derived from monitoring

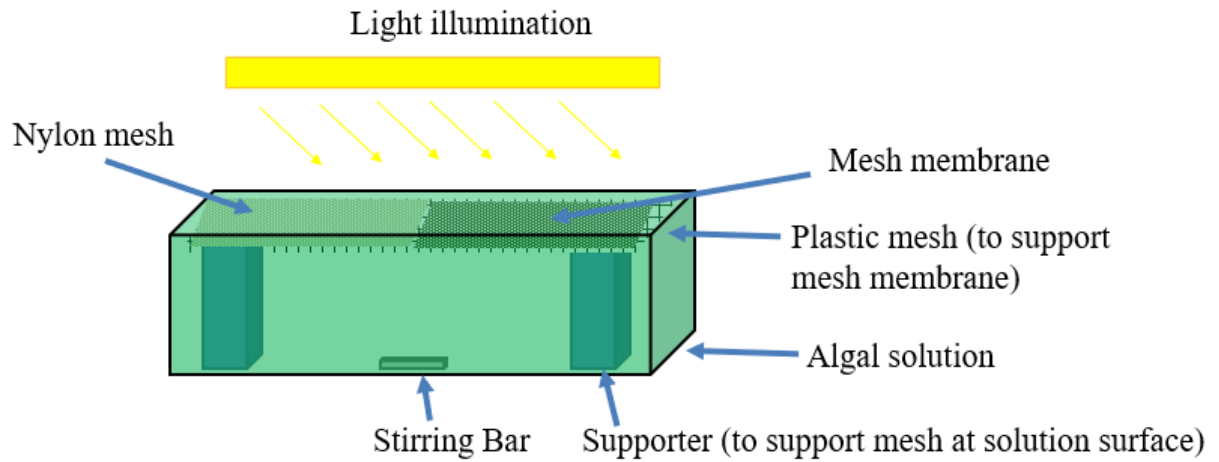


Figure H.1 Schematic of a test tank to inoculate the algal seeds onto specific MM (polyester or nylon mesh with various pore sizes) in the test of optimal MM material and pore size.

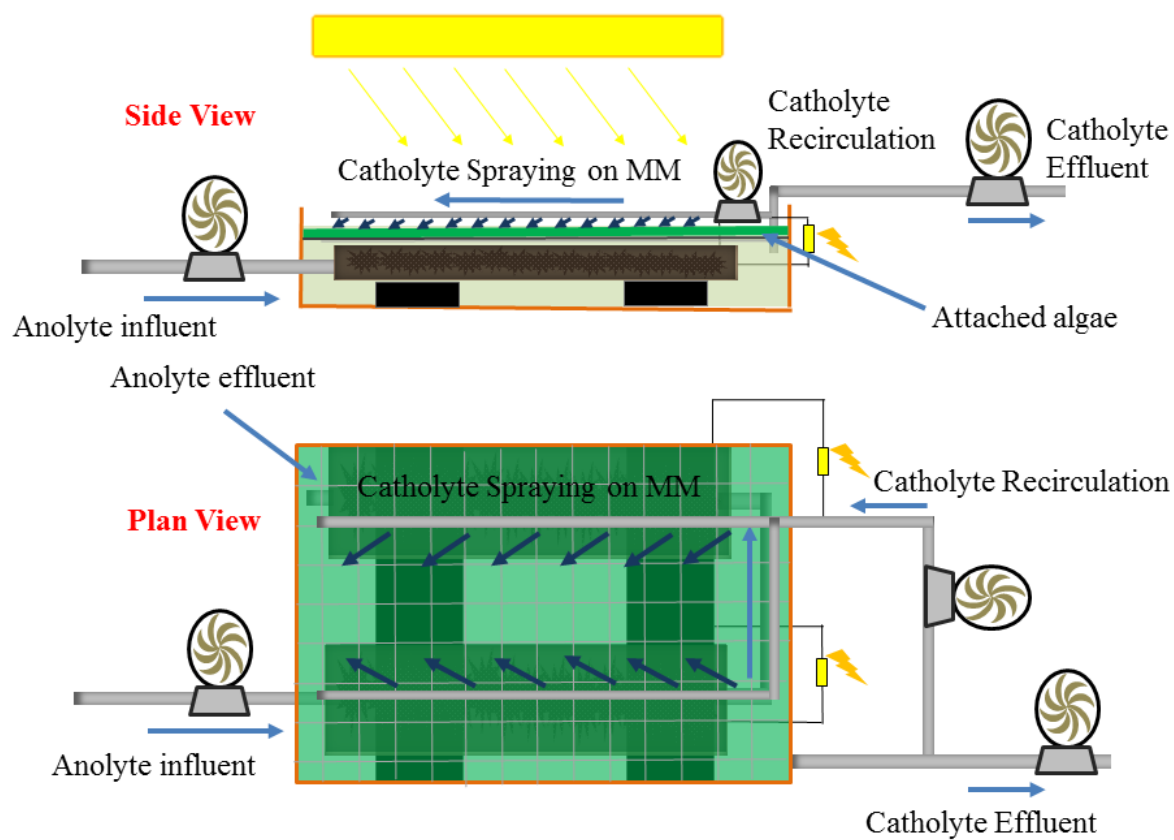


Figure H.2 System schematic of the IPB system with catholyte recirculation that returned the catholyte to the top of MM.

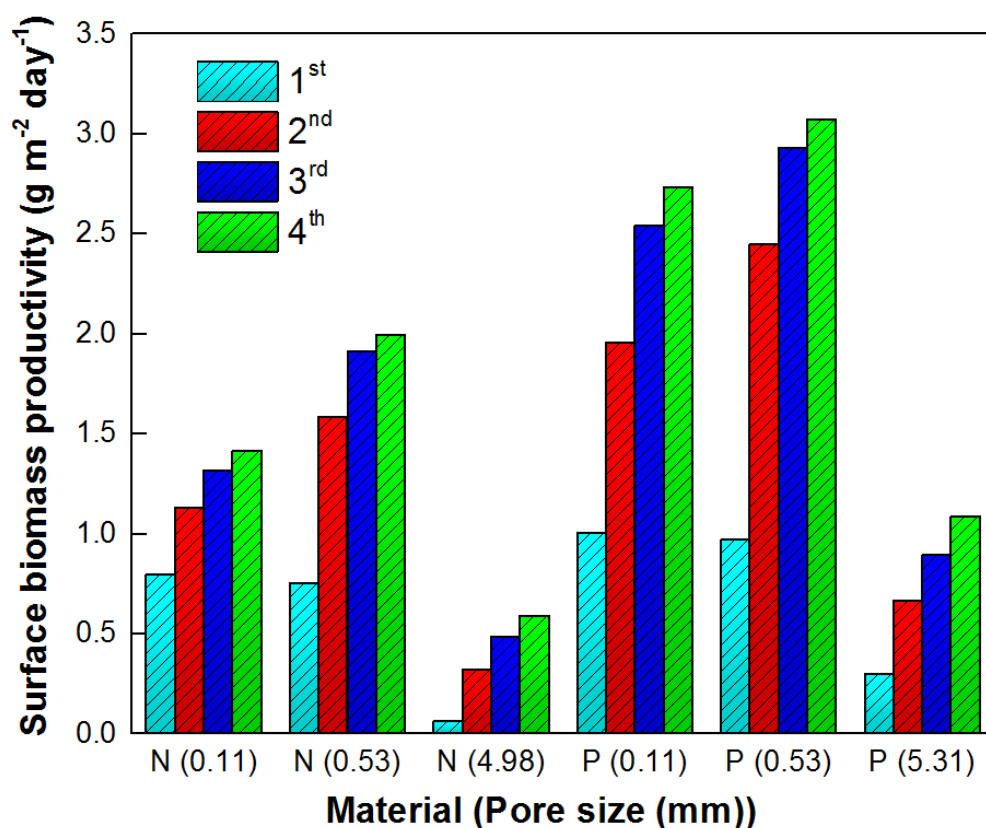


Figure H.3 Surface biomass productivity (SBP, g m⁻² day⁻¹) for all six different MM materials from 1st to 4th cycle after placing the MM into the algal cultivation tank (initial algal suspended concentration at 1 g L⁻¹). Note: “N” represents nylon; “P” represents polyester; the number in parenthesis represents the pore size (mm) of the MM in the algal tank.

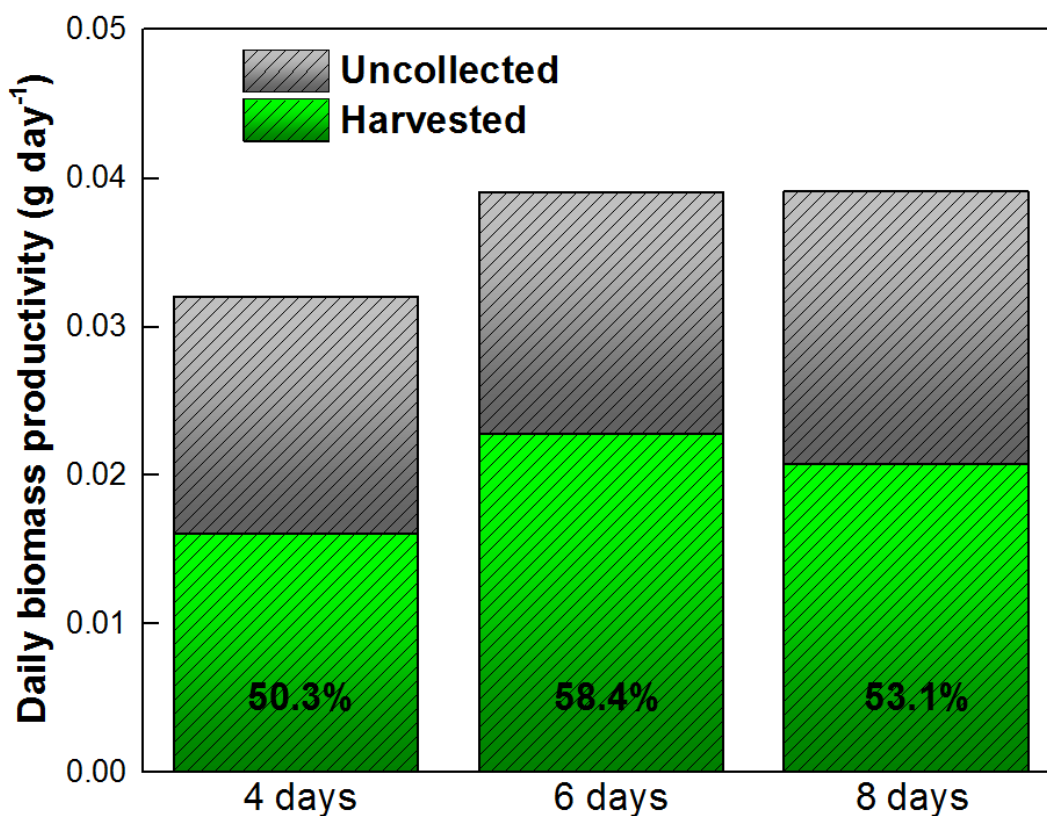


Figure H.4 Daily biomass productivity (g day⁻¹) and algal harvesting efficiency (%) of IPB system throughout the entire experimental period (including three cycles) with various algal growth period at 4, 6 and 8 days under only 1 harvesting time in a cycle. Note: the black number in the column indicates the harvesting efficiency (%).

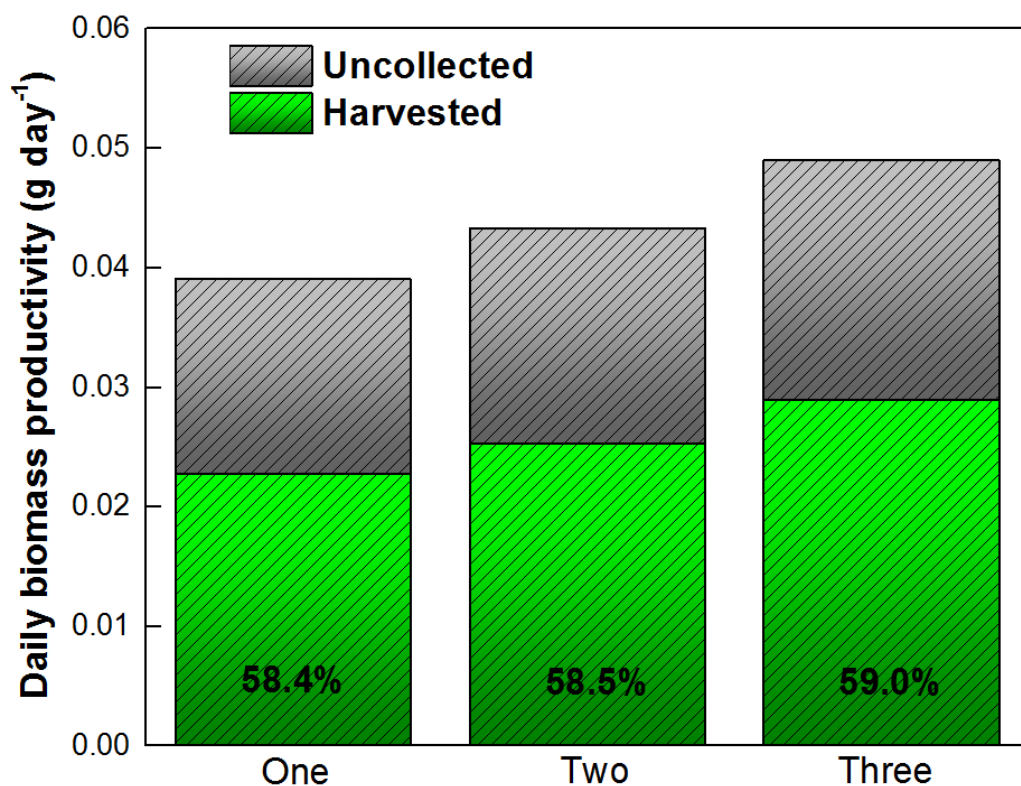


Figure H.5 Daily biomass productivity (g day⁻¹) and algal harvesting efficiency (%) of IPB system throughout the entire experimental period (including three cycles) for using various algal harvesting times at 1, 2 and 3 with the same algal growth period at 6 days in a cycle. Note: the black number in the column indicates the harvesting efficiency (%).