Chapter 2
Role of Bioreactors in Microbial Biomass and Energy Conversion

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1 Introduction

In recent decades, the global energy crisis and widespread environmental destruction caused by over-exploitation of traditional, petroleum-based energy sources have put forward a pressing need to develop renewable and sustainable energy. Renewable sources of energy, including biomass, hydropower, geothermal, solar, wind and marine energy, have become important and promising parts of the energy infrastructure. Among the different types of renewable energy, bioenergy is a widely available source that supplies combustion for motor fuels, electricity power, and other domains. Bioreactors are the principal devices that provide a suitable environment for the biochemical reactions involved in microbial biomass cultivation and energy conversion. Bioreactor technology attracts great interest in processes such as microbial biomass cultivation, microbial biofuel conversion, and microbial electrochemical systems because of its simplicity, moderate reaction, sustainability, low energy and raw material input, and minimal carbon footprint. Undoubtedly, bioreactor technology is one of the most promising approaches for microbial biomass production and energy conversion, and plays a significant role in

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this bioprocess. In this chapter, the role of bioreactors is reviewed with respect to its application in microbial biomass cultivation, microbial biofuel conversion, and microbial electrochemical systems. In addition, fundamental aspects and noteworthy functions of bioreactors are outlined within each of these applications.

2 Bioreactors in Microbial Biomass Cultivation

It is well known that microbial energy conversion technology is an effective and eco-friendly solution to global energy problems. Within this technology, cultivation of microbial biomass such as microalgae is one of the most promising. The lipid productivity of microalgae is ten times higher than that of terrestrial crops like soybean and sunflower. Additionally, microalgae have many other advantages like high photosynthetic efficiency, low water footprint, and the ability to grow on non-arable land using nutrients from wastewater. The microbial energy conversion process is usually conducted in a bioreactor, which provides a suitable environment for microbial cells. Therefore, bioreactors play a significant role in the cultivation of microbial biomass and can promote the widespread use of bioenergy.

2.1 Application in Microbial Biomass Cultivation

As one of the main methods of producing microbial biomass, microalgae cultivation can be divided into photoautotrophic, heterotrophic and mixotrophic cultivation depending on the type of carbon and energy source.

In photoautotrophic cultivation, microalgae use photosynthesis to convert light and inorganic carbons (e.g., CO_2 and HCO_3^-) into high-value organic matter, which is nature's most primitive way of converting carbon into biomass. The synthetic intracellular organic matter produced by microalgae in photoautotrophic cultivation can be made into biodiesel, cosmetics, and food. Additionally, photoautotrophic cultivation is suited to large-scale production due to its easy, low-cost operation and maintenance. However, photoautotrophic culture medium impedes light penetration, thereby greatly restricting high-density microalgae cultivation. At present, the microalgae concentration in small photobioreactors can reach 5–6 g/L, but the concentration in outdoor ponds only reaches 1–1.5 g/L. The low density of microalgae makes it difficult to separate the useful biomass from excess bulk and limits the large-scale cultivation of microalgae in bioreactors.

In heterotrophic cultivation, microalgae utilize organic carbon sources present in the culture medium (like glucose and acetic acid) to synthesize intracellular macromolecules like chlorophyll, proteins, and carbohydrates, without the need for light energy [1]. Heterotrophic microalgae cultivation can produce a relatively high biomass concentration since it directly utilizes organic matter in the medium as the energy source, thereby avoiding the growth limitations caused by insufficient light

penetration in photoautotrophic cultivation. It is reported that the biomass concentration of heterotrophic cultivation can easily reach 100 g/L. Therefore, in terms of high-density accumulation of biomass, heterotrophic cultivation of microalgae in bioreactors is superior to photoautotrophic cultivation [2]. However, there is an increased risk of bacterial contamination in heterotrophic cultivation, requiring the culture medium to undergo a thorough sterilization process prior to use. Additionally, the cost of replenishing the culture medium is also an important factor hindering commercial heterotrophic production of microalgae biomass [3].

In mixotrophic cultivation, microalgae cells are capable of reproducing under both photoautotrophic and heterotrophic conditions, using both inorganic and organic carbons as their energy source for growth [4]. Although mixotrophic cultivation can partially reduce the photo-limitation effect present in photoautotrophic cultivation and the bacterial contamination risk present in heterotrophic cultivation, it is rarely used in large-scale microalgae biomass and biofuel production due to operating complexity.

2.2 *Bioreactors in Microbial Biomass Cultivation: The Fundamentals*

A bioreactor refers to any manufactured or engineered system that supports a biologically active environment [5]. A photobioreactor (PBR) is a type of bioreactor that incorporates a light source (natural sunlight or artificial illumination). The success of mass production of microalgae for biodiesel depends greatly on the design and performance of PBRs [6].

2.2.1 Configurations

As shown in Fig. 1, bioreactors for microalgae cultivation can be categorized into open ponds (raceway pond) and closed PBRs (flat-plate, column, and tubular PBRs) [7].

Open ponds can be subdivided into natural waters (lakes, lagoons, and ponds), artificial ponds, and containers [8]. Because of their low cost, convenient maintenance, and large-scale suitability, open ponds are widely used in commercial production. Taking raceway ponds as an example, the depth of microalgae suspension is about 15–30 cm, with circulating flow driven by a pump. This water circulation keeps the cells in suspension, generating enough velocity to prevent cells from settling or aggregating via flocculation. However, the major limitations in open ponds include poor productivity, the need for large tracts of land, the restriction to certain microalgae strains, poor light utilization, and constant loss of water via evaporation [9]. Raceway ponds can typically yield a biomass concentration of 1 $g_{dryweight}$/L and reach a productivity level of 100 $mg_{dry\ weight}$/L/d [10].

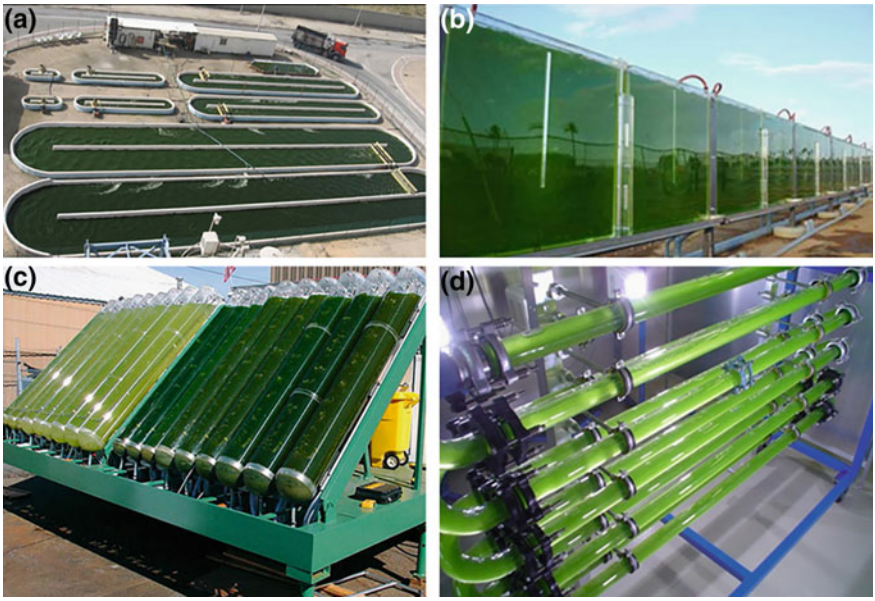

Fig. 1 Raceway pond (**a**), flat-plate photobioreactor (**b**), column photobioreactor (**c**) and tubular photobioreactor (**d**) (adapted and reprinted from [7], Copyright 2011, with permission from Elsevier)

A closed PBR is generally made of glass, plastic, or some other transparent material [11]. Since the culture conditions (like temperature, humidity, and flow parameter) are easy to control, this kind of bioreactor has some advantages over open ponds, such as large illuminated surface area per unit volume, long gas residence time, high growth rate, and high biomass concentration. In addition, the closed PBR is less likely to cause environmental pollution. Therefore, extensive research is focused on optimizing the closed PBR. In terms of structure, closed PBRs can be divided into three types: the flat-plate, the column, and the tubular PBR [7]. Flat-plate PBRs have attracted particular interest for the cultivation of photosynthetic microorganisms due to their large illuminated surface to volume ratio and their modular design that is convenient for scaling up. For example, Pulz et al. [12] reported an optimized, large-scale, flat-plate PBR module of 6000 L, and Tredici et al. [13] developed a vertical alveolar panel (2.2 m^2) based on the same material. In column PBRs, CO_2 gas is bubbled into the microalgae suspension through a gas distributor to provide the carbon source for growth. The force of the rising bubbles keeps the microalgae suspension circulating. The advantages of column PBRs include efficient mass transfer, good mixing with low shear stress and low energy consumption [9]. Tubular PBRs are usually made of transparent materials and are placed under natural sunlight or an artificial light source. The mixing of the microalgae suspension in this type of bioreactor is driven by a pump or airlift system. Some disadvantages include high manufacturing cost,

poor circulation, and accumulation of dissolved oxygen [14]. Nevertheless, tubular PBRs are currently the most widely used type of closed bioreactor for large-scale cultivation of microalgae due to their large illuminated surface to volume ratio and relatively high biomass productivity.

2.2.2 Functions

There are four main applications of bioreactors in microalgae cultivation [15]:

(1) Bioreactors are used to cultivate small phototrophic organisms, such as cyanobacteria, algae, or moss plants for biodiesel and other liquid fuels [16]. For example, microalgae open pond systems were used for biofuel production between 1980 and 1996 by the United States (U.S.) Department of Energy's ASP plan [17].
(2) Bioreactors can be used to generate microalgae biomass and other high-value metabolites. The nutrient composition of algal biomass has proved to be a superior alternative in animal feed supplements [18]. For example, the most common *Chlorella* sp. and *Spirulina* sp. can be used as food, food additives, and feed supplement in the form of algal biomass powder.
(3) Bioreactors are also designed to treat exhaust gas and wastewater [19]. Since CO_2 is efficiently absorbed by microalgae in bioreactors, flue gas and other industrial waste gases can be used to provide their carbon source. Microalgae cultivated in bioreactors are used to absorb nitrogen, phosphorus, heavy metals, and other toxic substances present in wastewater [9].
(4) Bioreactors are used in manned space exploration programs as bioregenerative technology like the Controlled Ecological Life Support System.

2.2.3 Influencing Factors

Light is used as an energy source in microalgae photosynthesis, and light intensity is one of the most important factors in this process [20]. There are three aspects of light that influence the growth of microalgae: light intensity, optical wavelength, and photoperiod. Light is the driving force of photosynthetic electron transfer, and the electron transfer rate is improved with increased light intensity. An increased electron transfer rate in turn enhances the rate of photosynthesis. However, light intensities above the light saturation point can inhibit the growth of microalgae [21]. Therefore, in the process of microalgae cultivation, light intensity should be maintained as close to the light saturation point as possible.

CO_2 is the main carbon source for microalgae photosynthesis, and the CO_2 transfer process directly affects photosynthesis, especially the dark reactions of photosynthesis. According to the two-film theory, CO_2 molecules in the gaseous phase are utilized by microalgae cells in two main stages. In the first stage, CO_2 molecules transfer from the gaseous phase to the liquid phase. In the second stage,

this liquid form of inorganic carbon is converted into organic carbon in the microalgal cell [22]. Microalgae cultivation in an airlift PBR uses a typical carbon-capture process in which CO_2 is bubbled into the reactor via a gas distributor. As the gas rises up through the reactor, the CO_2 molecules diffuse across the gas-liquid interface and dissolve into the surrounding microalgae suspension. Afterwards, the dissolved CO_2 is consumed by microalgae cells to produce organic matter through photosynthesis [6, 23]. In the above-mentioned process, the efficiency of CO_2 delivery can significantly affect microalgae growth, bio-oil accumulation, and CO_2 fixation in the PBR.

Temperature is another important factor influencing microalgae metabolism by affecting enzyme activity. The optimum temperature range for microalgae growth varies by strain. In general, the ideal temperature for microalgae cultivation ranges from 20 to 24 °C. The most commonly used microalgae species can tolerate temperatures between 16 and 27 °C. Temperatures lower than 16 °C will attenuate the growth of microalgae, whereas temperatures higher than 35 °C are lethal for a number of species [24].

The pH of the culture medium has a large impact on microalgae growth and other physiological processes. The pH can affect the dissolution and diffusion of CO_2 in the liquid phase, thus affecting the efficiency of photosynthetic carbon fixation. In addition, the pH can influence microalgae respiration rate, ion absorption, metabolism, and distribution of algal cells in the bioreactor [25–27]. Different species of microalgae prefer different pH values. Additionally, the pH of a microalgae suspension tends to increase during the cultivation process, which hinders the enzymatic activity of the microalgae and in turn inhibits growth. Therefore, it is important to actively maintain the pH of the culture medium at the optimum range by adding acetic acid or hydrochloric acid to the medium during cultivation.

2.3 *The Importance of Bioreactors in Microbial Biomass Cultivation*

2.3.1 Ideal Site for Microbial Growth and Metabolic Reactions

Bioreactors are the primary devices that provide a suitable environment for the biochemical reactions mediated by microorganisms. Almost all microbial metabolic processes are carried out in bioreactors. Growing microorganisms in bioreactors reduces the risk of contamination, improves the reproducibility of cultivation conditions, provides controlled hydrodynamics, temperature, and substratum, and allows appropriate technical design [28]. Bioreactors provide favorable physical and chemical environments for biological metabolism, allowing microorganisms to grow and metabolize at relatively high rates. In the biological engineering industry, the bioreactants, substrate, enzyme, catalyst, and nutrients are added to the bioreactor to undergo biochemical reactions aided by microbial cells. Then, these microbial cells

proliferate and synthesize various metabolic products. Bioreactors provide the necessary mixing, mass transfer, and physical containment to guarantee a controlled environment for the organism to produce the desired biological product [29].

During the past few decades, bioreactors have been applied in many fields for biomass cultivation and metabolite production. Chinnasamy et al. [30] used open and closed bioreactors for microalgae biomass accumulation. Lehr and Posten [31] employed PBRs to produce biofuel. Zhang et al. [32] analyzed the performance of a groove-type PBR for hydrogen production by immobilized photosynthetic bacteria. Pen et al. [33] designed an innovative membrane bioreactor for methane biohydroxylation. Furthermore, bioreactors have been widely used in many fields such as the chemical, pharmaceutical, material, environmental protection, and metallurgy industries. The development and optimization of bioreactors and their operating parameters are therefore a key focus of biochemical engineering.

Microbial biomass production involves a series of complex biochemical reactions that call for specific physicochemical properties of the reactants and appropriate transfer characteristics of light, mass, and heat [34]. A high solid liquid ratio is conducive to the mass and heat transfer process in microbial systems. The viscosity, turbidity, and homogeneity of the reaction liquid inside the bioreactor, as well as its physical properties (temperature, light intensity, pH, etc.), have important influences on the biomass production process. In other words, flow pattern, light penetration properties, as well as heat and mass transfer characteristics of the reaction liquid directly influence the microbial biomass production process.

2.3.2 Multiphase Flow

For the optimal design and operation of bioreactors, turbulent mixing of multiphase flows have been recognized as an important factor in determining the overall performance of the bioreactor [35]. In a well-mixed bioreactor, local turbulences can position the microorganism's cells randomly throughout the container and near the substratum sources. As a result, each individual cell is exposed to a suitable growth environment. A well-mixed bioreactor is also necessary to prevent cells from settling or attaching to the reactor walls. Free-floating cells allow for maximal gas exchange and prevent accumulation of excess metabolites that could inhibit growth. However, intense mixing can inhibit growth by causing high levels of shear stress and physical damage to the cells [36]. Therefore, appropriately controlled circulation is a key factor for the optimal design and operation of a bioreactor.

Several studies have investigated the optimum circulation dynamics of different types of bioreactors. Ninno and Power [37] investigated turbulent multiphase flows in a flat-panel bioreactor and their consequent effects on microalgae cultivation using Computational Fluid Dynamics (CFD) simulation and Particle Image Velocimetry (PIV). Liao et al. [38] conducted Lattice Boltzmann simulation on liquid flow and mass transfer in a bioreactor with a cylinder bundle for hydrogen production. Sikula et al. [39] developed a fermentation model in an internal loop

airlift bioreactor to enhance fermentation efficiency. Zhang et al. [40] studied multiphase flow in an anaerobic bioreactor with a multi-scale approach.

2.3.3 Heat and Mass Transfer

Heat and mass transfer in a bioreactor mainly include the transfer of light, heat, organic or inorganic matter, and metabolites. Most bioreactions are sensitive to temperature. Hence, it is essential to analyze the thermal dynamics of the microbial biomass production process and regulate heat transfer to achieve efficient biomass production.

Laukevics et al. [41] reported that steric hindrance, which encompasses the effects of geometry, mass transfer, and substrate availability, was partially responsible for limited microbial growth in the void spaces of the substrate bed. Rathbun and Shuler [42] reported that steep temperature gradients (reaching 50 °C in the reactor bed) inside the static bioreactor were sufficient to prevent the growth of microorganisms. The interaction between the complex phenomena of heat and mass transfer often leads to the development of steep concentration and temperature gradients, resulting in non-homogeneous conditions in the bioreactor and subsequent low efficiency. In addition, Rajagopalan and Modak [43] modeled the heat and mass transfer for solid-state fermentation (SSF) in a tray bioreactor. The extent of growth restriction due to inefficient heat and/or mass transfer was analyzed during different stages of fermentation. It is expected that this model can lead to a better understanding of the transport processes in SSF, thereby allowing optimization of bioreactor design for SSF. A different study by Valiorgue et al. [44] investigated CO_2 mass transfer and conversion to improve microalgae biomass accumulation in a horizontal gas-liquid PBR.

2.3.4 Energy Conversion

The potential for the production of biofuels or other valuable byproducts from biomass has recently been intensively investigated. However, many bottlenecks still exist and most of these processes are considered cost-prohibitive [45]. Open PBR systems like raceway ponds have proven to be the most cost-effective method to produce microbial biomass on a large scale but closed PBR technologies are still necessary to provide inoculum cultures for the large-scale systems [46]. Even for the production of high-value products, the cost of manufacturing and operating closed bioreactors can be restrictive. Therefore, there is much ongoing research examining and optimizing the cost-benefit ratio of PBRs. Jacoblopes et al. [47] investigated the biotransformation of carbon dioxide (CO_2) in PBRs. Murphy [48] designed an artificial leaf for biofuel production and improved the energy conversion efficiency in the bioreactor system. Moreover, many researchers have investigated the light energy conversion process and designed many kinds of novel bioreactors to improve the efficiency of energy conversion [49–52].

3 Bioreactors in Microbial Biofuel Conversion

Over-utilization of traditional fossil fuels has caused severe energy shortages and environmental damage. The combustion products of fossil fuels, like SO_2, NO_x, CO, CO_2, etc., are harmful to the environment. It is therefore necessary to develop renewable and environment-friendly energy sources [53]. Microbial biofuels are promising energy alternatives owing to prevalent raw materials, mild operation conditions, and clean combustion products. Currently, increasing efforts are being made to improve biofuel production using this promising approach. In order to maximize biofuel production, microbial cells require an optimal environment for growth. To maintain such favorable conditions for microbial growth and metabolism, bioreactors play an indispensable role.

Bioreactors, especially closed bioreactors, can provide the ideal milieu for microbial growth and metabolism. Microbial biofuel conversion is mainly divided into an upstream treatment process that includes fermentation for microbial growth and product generation, and a downstream treatment process that includes product purification, isolation, and collection [54]. In order to improve energy conversion efficiency, the specifications of the bioreactor should integrate not only the correct structural configuration but also precise operational control for optimized multiphase flow as well as heat and mass transfer in the reaction solution.

3.1 Application in Microbial Biofuel Conversion

Due to their adaptable operating conditions, bioreactors are widely used in different types of microbial biofuel conversion processes, such as biogas production by anaerobic digestion, hydrogen production by photo-fermentation or dark-fermentation, alcohol production by fermentation, and fatty acid production by microalgae. Microbes utilize a variety of substrates (cellulose, hemicellulose, starch, glucose, xylose, etc.) to produce biofuels. During the biofuel conversion processes, microbial cells are sensitive to variations in their surroundings, and any instability is detrimental to their growth and product synthesis. In bioreactors, the environmental parameters (temperature, pH, medium composition, retention time, mass and heat transfer rate, etc.) can be maintained at near-optimal ranges to enhance microorganism growth and product accumulation.

Anaerobic digestion is a biological process in which microbes metabolize organic substrates in the absence of oxygen to produce biogas [55]. This biogas is composed of a mixture of compounds, with methane and CO_2 as major contributors. Fermentative hydrogen production is an anaerobic reaction that has received substantial attention in recent years owing to advantages such as rapid hydrogen production rate, mild production conditions, and ease of operation. There are two

types of fermentative hydrogen production: photo-fermentative production and dark-fermentative production. Photo-fermentative hydrogen production is mainly catalyzed by photosynthetic bacteria, whereas dark-fermentative hydrogen production is mainly catalyzed by anaerobic bacteria. Alcohols and fatty acids can also be produced via anaerobic fermentation processes. Both alcohols and fatty acids are potential substitutes for petroleum-derived fuel as they have comparable properties to those of gasoline [56]. Types of alcohols that can be generated by fermentation include short-chain alcohols like ethanol and several higher alcohols like 1-butanol, isobutanol, 2-methyl-1-butanol and 3-methyl-1-butanol [56]. The most widely used form of alcohol is ethanol; however, being highly corrosive and hygroscopic, it is not conducive to the existing fuel storage and distribution equipment [57]. On the contrary, n-butanol, isobutanol, and other higher alcohols have a lower hygroscopicity compared to ethanol, with an energy density (27 MJ/L) close to that of gasoline (32 MJ/L) [58]. In addition, these higher alcohols are compatible with the existing fuel storage and distribution infrastructure. Fatty acids can be classified into three groups by the length of their carbon chains, i.e., short-chain fatty acids (less than 6 carbon atoms), mid-chain fatty acids (6–12 carbon atoms) and long-chain fatty acids (more than 12 carbon atoms) [59].

3.2 Bioreactors in Microbial Biofuel Conversion: The Fundamentals

A bioreactor represents the equipment in which biological reactions and microbial cell reproduction occur using enzymes or living cells as biocatalysts. The bioreactors can simulate the biological characteristics and physiological functions of microbial cells and tissues to synthesize target products. They are widely used in a variety of fields, like food and agriculture, health and medicine, energy, and environmental protection. In particular, bioreactors play an important role in microbial biofuel conversion where microbial catalysts generate biofuels like biohydrogen, biogas, alcohols and fatty acids. Research has developed various configurations of bioreactors with optimized operating conditions to maximize biofuel output [60]. The merits and limitations of each type of bioreactor are discussed below.

3.2.1 Configurations

Microbial biofuel conversion is a complex biochemical process that is greatly dependent on the configuration of the bioreactor where it occurs. Bioreactor design is usually conducted on an experimental basis, taking into consideration influencing factors like gas-liquid-gas multiphase flow, mass and heat transfer balance, and energy conversion efficiency. A bioreactor with superior performance requires a

watertight structure, high heat and mass transfer efficiency, good mixing performance, low energy investment, and high product output. Thus far, the most commonly used configurations include: (i) conventional anaerobic reactors such as the anaerobic sequencing batch reactor, the continuous stirred tank reactor, and the anaerobic plug-flow reactor; (ii) sludge retention reactors such as the anaerobic contact reactor, the up-flow anaerobic sludge bed reactor, the up-flow anaerobic solid-state reactor, the anaerobic baffled reactor, and the internal circulation reactor; and (iii) anaerobic membrane reactors such as the anaerobic filter reactor, the anaerobic fluidized bed reactor, and the expanded granular sludge blanket.

The conventional anaerobic reactor is a single-tank system that utilizes the same tank for substrate treatment and fermentation [61]. All steps of microbial biofuel conversion take place in a single tank, which means that downstream treatment processes as well as the intermediate byproducts can have significant negative influences on the upstream treatment processes. Thus, efficient approaches to avoid the interactive effects of different reactions are essential to enhance bioreactor performance.

The configuration of sludge retention reactors is relatively complex compared to the conventional reactors. Sludge retention reactors usually contain two main components: the liquid-phase reaction module and the solid-phase recycling or gathering module. For example, the anaerobic contact reactor contains an agitated reactor module and a solid phase setting module to recycle the microorganisms, whereas the up-flow anaerobic sludge bed reactor contains the liquid-phase reaction module at the top of the reactor and a dense sludge bed located at the bottom of the reactor. Sludge retention reactors provide good contact between wastewater and biomass, which prevents washout of microorganisms. They are often used to process effluents containing high concentrations of suspended solids.

Anaerobic membrane reactors are constructed with a supporting membrane to enhance contact between wastewater and the bacterial microorganism. The bacterial biofilm accumulates and grows on this supporting membrane, causing a separation between bacterial biomass and the wastewater in the reactor. For example, the anaerobic filter reactor contains a filter on which the bacterial biofilm grows. In the anaerobic fluidized bed reactor, inert particles like fine sand and alumina are provided for the thin bacterial biofilm to grow on. The configurations of anaerobic membrane reactors enhance the resistance of the microbes to inhibitors, thereby improving biofuel production.

3.2.2 Functions

In the microbial biofuel conversion process, bioreactors provide fine control of operating conditions for microorganism growth, metabolism, and product synthesis, thus improving biofuel production. For example, the pH can be maintained at suitable levels by adding buffer solutions, the temperature can be controlled by a thermostatic water bath, and the hydraulic retention time (HRT) of wastewater can be controlled by regulating the inward feeding rate. The structural configuration of a

bioreactor closely aligns with its functional advantages. Different structural characteristics are required for different applications of a bioreactor. For example, the leakage resistance of a bioreactor is critical when applied to biogas production. The function of conventional anaerobic reactors is to supply relatively stable operating conditions in an established temporal sequence. Owing to its simple structure, the sequencing anaerobic reactor has advantages of operational simplicity and low cost. However, the self-immobilization of the conventional anaerobic reactor is poor, and the channeling and clogging effects severe. These disadvantages limit reactor performance and biofuel conversion efficiency. The major function of sludge retention reactors is recycling of microbial biomass, thus avoiding biomass washout. These reactors rapidly achieve steady-state due to short hydraulic retention time [62]. In addition, some configurations of sludge retention reactors can have special functions. For example, in the anaerobic baffled reactor, the flow patterns of waste influents can be regulated by arranging the baffles, serving to separate acidogenesis and methanogenesis along the vertical axis of the reactor and allowing different bacterial communities to develop under independently suited conditions [63]. The function of the anaerobic membrane reactor is based on the supporting membrane material used for microbial biofilm formation, which serves to separate influents from microbial biomass. By generating this microbial biofilm, biomass washout can be avoided and the microbes have a longer retention time than the hydraulic retention time. As a result, the mechanical mixing and sludge settling that occur in sludge retention reactors can be avoided in anaerobic membrane reactors [64].

3.2.3 Influencing Factors

During the microbial biofuel conversion process, product yields are affected by many factors including temperature, pH, nutrient content, organic loading rate, type of reactor, hydraulic retention time and solids retention time [61, 65, 66]. In particular, bioreactor design is important for applications of biochemical engineering. The design of a bioreactor includes determination of operating conditions, reactor size, mixing and mass transfer capabilities, temperature and sterility conditions, the means of feed introduction and product removal, and control of operating variables such as pH, oxygen concentration, and illumination [67]. Reactor size and shape usually influence biofuel output capacity. Increasing the size of the container can improve biofuel production to some extent, but can also cause biomass concentration gradients in the reactors, which hinders biofuel production. Bioreactors operated at low temperature are less prone to thermal instability and degradation. However, since some thermophilic bacteria prefer high ambient temperatures of up to 65 °C, bioreactors must maintain the standard for thermotolerance. Generated byproducts can dissolve and accumulate in the bioreactor over time, inhibiting microbial growth and metabolism. Thus, in order to maximize the efficiency of microbial biofuel conversion, bioreactor design must incorporate some mechanism to quickly remove such byproducts.

3.3 The Importance of Bioreactors in Microbial Biofuel Conversion

3.3.1 Ideal Site for Microbial Metabolic Reactions

By appropriately controlling the operating conditions, bioreactors provide a near-optimal environment for biofuel conversion reactions. Inside the bioreactor, biogas is produced via the degradation of organic substrates, cycling consecutively through hydrolysis, acidogenesis, acetogenesis, and methanogenesis [68]. Firstly, the fermentative microbes excrete hydrolytic enzymes that break down complex carbohydrates, lipids, and proteins into soluble monomers and oligomers, long-chain fatty acids, and amino acids, respectively. Then, these soluble molecules are converted into volatile fatty acids, CO_2, alcohols, and hydrogen by acidogenic microbes. Next, the volatile fatty acids and alcohols are converted into acetic acid and hydrogen via acetogenesis. Finally, the acetic acid, CO_2, and hydrogen are converted into methane through acetotrophic, hydrogenotrophic, and methanogenic reactions [69]. A bioreactor designed to incorporate the physiological requirements of several different microbial communities (acidogenic bacteria, acetogenic bacteria, and methanogenic bacteria) is necessary to maximize biogas production from anaerobic digestion.

In photo-fermentative hydrogen production, the degradation of organic substrates generate electrons that need to be eliminated to maintain electrical neutrality of the system [70]. Surrounding protons act as electron acceptors, thereby generating hydrogen (H_2). Fermentative hydrogen production can be described by Eq. (1) when microorganisms use glucose as the organic substrate. The glucose is first converted to pyruvate and NADH (the reduced state of nicotinamide adenine dinucleotide) via the glycolytic pathway. Then, the pyruvate is converted to acetyl coenzyme A (acetyl-CoA), CO_2 and H_2 by pyruvate-ferredoxin oxide-reductase and hydrogenase enzymes [65, 66].

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2 \quad (1)$$

To produce alcohols, microorganisms first reduce primary metabolites like acetyl-CoA and pyruvate and then elongate them into electron-rich compounds like higher carbon acyl-CoA and 2-keto acids. Then the electron-rich compounds are further reduced into higher alcohols by microbial metabolism and secreted into the medium [58]. Similarly, fatty acids are produced through an anaerobic process involving hydrolysis and acidogenesis. During hydrolysis, complex organic polymers are broken down into simple monomers by specific enzymes. Subsequently, during acidogenesis, acidogens ferment the monomers into fatty acids (acetic acid, propionic acid, butyric acid, etc.). Fatty acids can also be produced by photosynthetic bacteria (like algae and cyanobacteria) through photosynthesis. All these microbial biofuel conversion reactions are conducted in bioreactors and are discussed in the following text.

3.3.2 Biomass Cultivation

Another important role of the bioreactor is to provide a suitable environment for microbial reproduction, namely microbial biomass cultivation. Biofuel production is collectively determined by both microbial biomass concentration and specific biofuel production capacity. A bioreactor with high biofuel production requires high biomass concentration as well as high specific biofuel productivity. However, the robust growth of microbes and high specific biofuel productivity are mutually exclusive, especially for microalgal lipid production [71]. When microalgae are cultivated in optimal conditions for growth, the lipid content in the cells is usually poor [72]. Conversely, when microalgae are cultivated in optimal conditions for lipid synthesis, microalgae growth is usually slow. As a result, overall lipid productivity is rarely improved.

3.3.3 Multiphase Flow

Microbial biofuel conversion requires a typical multiphase flow system in which all three phases (gas, liquid and solid) coexist. The gases present in the bioreactor are mainly H_2, CO_2, and O_2 produced by the metabolism of microbes and algae, the liquid phase consists primarily of influents like wastewater, and the solid phase consists of the microbial biomass and particles suspended in the influents. The gases produced by microbial metabolism exist in the reaction solution as bubbles and rise to the top of the bioreactor by the force of buoyancy. The rising bubbles create a constant flow of the liquid solution and the microbial biomass, and the flow patterns are influenced by bioreactor shape and size. For example, the up-flow anaerobic sludge bed reactor permits upward circulation, whereas the anaerobic baffled reactor allows alteration of this flow pattern by adjusting the position of the baffles. Aside from the structural configuration of the bioreactor, mixing method can also alter multiphase flow characteristics of the system. An efficient mixing method is particularly important in photo-fermentative hydrogen production since mixing is beneficial for the effusion of hydrogen from the reaction solution and can prevent the inhibitory effect caused by hydrogen dissolution in the reaction liquid [73]. In conclusion, the flow characteristics and rheological properties of multiphase flow systems can significantly influence mixing and contact between the microbes and the liquid solution. They can also influence the heat and mass transfer between different phases in the bioreactor, which are important factors affecting the growth and metabolism of the microorganism.

3.3.4 Heat and Mass Transfer

Inside a bioreactor, heat and mass transfer characteristics of the reaction solution directly influence the microbial biofuel conversion processes. For example, dissolved hydrogen concentration (i.e., hydrogen partial pressure) negatively affects

the metabolism of photosynthetic bacteria by decreasing the mass transfer efficiency of hydrogen. In other words, when hydrogen concentration in the reaction solution is high, the mass transfer efficiency of hydrogen is reduced, hindering the productivity of the microbes [34]. Thus, removal of dissolved hydrogen from the reaction liquid by adjusting the operating conditions (temperature, pressure, etc.) is important to enhance mass transfer and subsequent hydrogen yield. Temperature and pressure can affect mass transfer characteristics by influencing the physical and chemical properties of the reaction solution. The surface tension of the reaction solution, density, viscosity, diffusion coefficient of the solute, and the individual properties of reaction byproducts also influence the mass transfer characteristics of the system [34, 74]. In general, mixing is the simplest way to enhance mass transfer efficiency, but the shear effect of the mixing wheels and the heat generated in the reaction liquid have a negative impact on microbial metabolism. Therefore, reduction of the shear effect and improvement of heat transfer are essential. There are many factors that influence the heat transfer efficiency of a bioreactor: the physical and chemical properties (thermal conductivity, specific heat, etc.) of the solution, the rheological properties (i.e., viscosity) of the reaction solution, flow characteristics of the solution, and ambient conditions (temperature, light intensity, etc.) [75]. Along with the running of the bioreactor itself, the heat produced by exothermic biochemical reactions gradually increase the internal temperature of the bioreactor. Thus, strategies to dissipate this accumulated heat by enhancing heat transfer and evaporative heat loss are important to keep the reaction solution at a temperature range conducive to maximal bacterial activity.

3.3.5 Energy Conversion

Transformation of energy sources (like organic substrates and light) to energy-containing products (biogas, hydrogen, fatty acids, alcohols, etc.) is a key purpose of a bioreactor. Researchers have explored several strategies to improve the conversion efficiency of the substrate or light energy. Maximal exploitation of the energy source is necessary to enhance energy conversion efficiency. For example, high light intensity, large illuminated surface area, and reasonable light distribution in bioreactors are effective methods to improve light conversion efficiency in the photo-fermentative hydrogen production process [34, 76]. However, excess light exposure significantly reduces light energy conversion efficiency by dissipating the light [77]. In theory, the highest light energy conversion efficiencies that can be achieved from microalgae biomass in the photo-fermentative hydrogen production process and in the lipid production process are 10% and 12%, respectively [78, 79]. But in reality, the actual efficiencies are very low due to light shading and scattering effects, which stand at about 8% in photo-fermentative hydrogen production [80] and 6% in lipid production via microalgae cultivation [81]. Similarly, the energy conversion efficiency of most fermentation processes is poor because the structure and composition of the fermentative substrates (with cellulose and hemicellulose components) are very complex. The conversion of cellulosic substrates to biofuels

requires deconstructing the robust outer structure to release the interior mass for further fermentation, and this step is a key limiting factor for high-energy conversion efficiency. Thus, researchers have proposed that cellulosic substrates undergo pretreatment methods to increase their surface area and porosity, while decreasing cellulose crystallinity and polymerization [82]. Commonly used pretreatment strategies include mechanical methods like milling and extrusion, thermochemical methods like pyrolysis, steam explosion, high pressure, and ammonia fiber explosion, physicochemical methods like alkali treatment, acid treatment, and gas oxidizing treatments, and biological methods like microbial deconstruction and enzymatic deconstruction [83]. Results from testing such methods showed that pretreatment of substrates can effectively improve substrate accessibility for energy conversion. For example, Dale et al. [84] demonstrated that hydrolysis yields in the reduction of sugar reached 90% of the theoretical yields after using the ammonia fiber explosion method to pretreat the lignocellulose substrate.

4 Bioreactors in Microbial Electrochemical Systems

Microbial electrochemical systems (MESs) exploit the metabolism of microorganisms to bio-electrochemically convert low-grade chemical energy stored in biodegradable substrates to high-grade energy (i.e., electricity) and value-added chemicals like hydrogen and methane [85]. As a rapidly evolving technology, MESs have been successfully implemented to treat wastewater for electricity generation (using microbial fuel cells; MFCs) and in biorefinery facilities (using microbial electrolysis cellss; MECs and microbial electrosynthesis; MEs). Specific applications include wastewater treatment [86, 87], power sources for remote sensors [88], research platforms for electrode-bacteria interaction [85, 89], and value-added component production [90–93]. Compared with other biological processes, MESs show higher versatility and lower sludge production [94], making them very promising in practical applications.

The substrates used in MESs can vary greatly from glucose, acetate, lactate, and dyes to domestic wastewater containing complex species [95]. Typically, these biodegradable substrates are electro-oxidized at the anode via bacterial metabolism to produce electrons and protons. Then, the electrons are conducted to the cathode and are accepted by oxygen, nitrate, or metal ions. After decades of research and development, the performance and stability of MESs have approached industry standards. It is predicted that MFCs can potentially produce 23.3 and 40 TWh of electricity from wastewater in India by 2025 and 2050, respectively [96]. Their long-term operational stability has also been verified. Zhang et al. [97] installed and operated two MFCs in a municipal wastewater treatment plant for over 400 days. The two MFCs showed great durability in COD (chemical oxygen demand) removal and fluctuation tolerance, demonstrating the long-term effectiveness of this technology outside the laboratory.

At the heart of an MES lies the bioreactor, where biodegradable substrates are converted to electrical current. The current is utilized directly (in MFCs) or conducted to the cathode for further reaction (in MECs). Therefore, the performance of an MES is dictated by the performance of the bioreactor within, where scientific disciplines like microorganism ecology, biomaterials science, mechanical engineering, and control strategy meet multiphysics phenomena like biofilm formation, multiphase flow, heat/mass transfer, and bio-electrochemical conversion.

This section aims to provide a fundamental and comprehensive overview of the bioreactors used in MESs, detailing principles in each transport phenomenon that play an important role in overall system performance. Perspectives on future development and optimization will also be discussed.

4.1 Application in Microbial Electrochemical Systems

For a typical wastewater treatment plant, about 45–75% of the energy needed is consumed by aeration treatment step [98]. In 2006, nearly 3% of all the electrical power produced in the U.S. ($\sim$110 TWh per year) was consumed by wastewater treatment [99]. Similarly, wastewater treatment constitutes up to 3–5% of the United Kingdom's national electricity consumption [100]. This demand is expected to increase further due to growing human population and higher environmental protection standards. If aspiring to the profitable operation of MESs, the energy cost has to be significantly reduced. On the contrary, wastewater has great potential for energy recovery; a report in 2004 indicated that domestic, industrial, and animal wastewater together contain $\sim$1.50 TWh of potential energy output in the U.S., which is higher than the power required for wastewater treatment [101]. Additionally, the energy-consuming aeration step can be replaced by MEs treatment; this would entirely eliminate aeration power consumption and allow the net power produced (10–20% of the aeration power) to be recycled for other processes within the MES. This strategy could make wastewater treatment commercially competitive.

Recently, Wang et al. reviewed the diverse applications of MESs, assessing 47 different functions and system configurations [85]. Figure 2 shows the basic principles of four typical MESs. Among these, MFCs are the most common application of MESs, converting the chemical energy stored in both the electron donor (i.e., the substrate) and electron acceptor (i.e., the oxidant) into electricity (Fig. 1a). A typical MFC consists of two chambers: the anaerobic anode chamber where microorganisms generate electrons via electro-oxidation of organic substrates and the aerobic cathode chamber where the reduction reaction takes place with oxygen or other chemicals. These two chambers are separated by an ion exchange membrane or a salt bridge to allow ion transport. Electrons produced by microorganisms are transferred to the anode by direct or indirect extracellular electron transport and

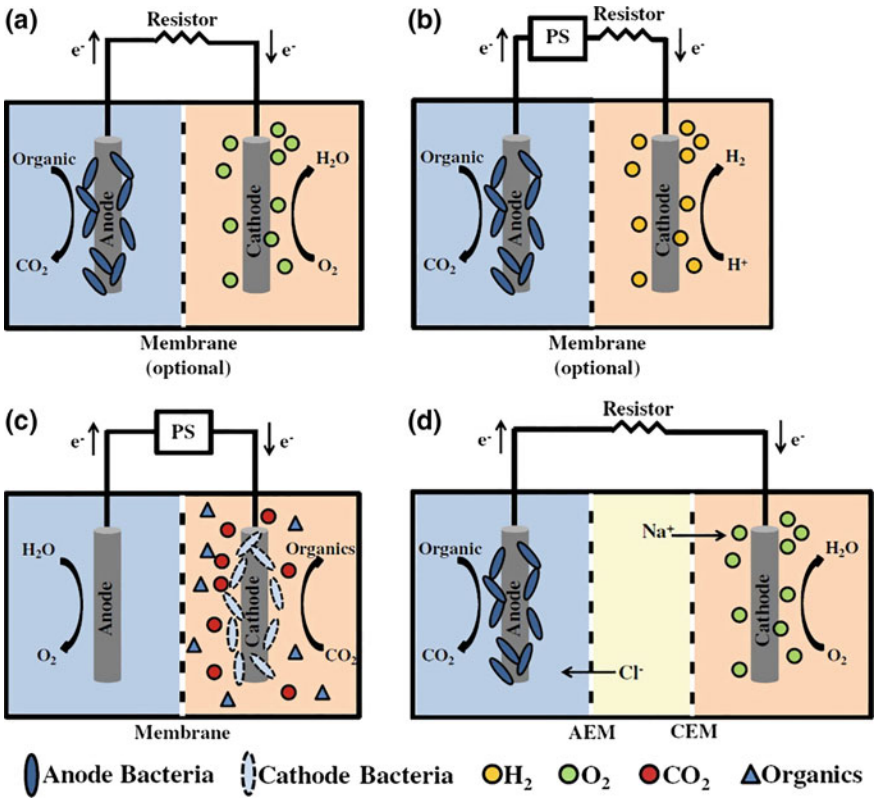

Fig. 2 Principles of four typical MESs (left chamber: anode; right chamber: cathode). **a** Electricity generation in air-cathode microbial fuel cells (MFCs); **b** hydrogen generation with external power supply in microbial electrolysis cells (MECs); **c** chemical production by microbial electrosynthesis (MES); **d** middle chamber desalination in microbial desalination cells (MDCs). (adapted and reprinted from [85], Copyright 2013, with permission from Elsevier)

are conducted to the cathode through an external circuit. Protons and other ions migrate through the membrane to complete the circuit. When an external power source is applied to increase the cathode voltage (0.6–1.0 V) for hydrogen production, the MFC is transformed into an MEC (Fig. 1b), where the mobile H^+ in the cathode chamber is reduced by the cathode to form H_2 or hydrogen gas [102]. This strategy provides a fundamentally new approach to hydrogen production that outperforms the traditional water-split (requiring a voltage higher than 1.2 V) and dark-fermentation methods in terms of power consumption and production rate. It was also noted that the produced H_2 can be further consumed by methanogenesis to produced methane (Fig. 1c) [102–104], especially in a single-chambered MES. In addition, the inner electrical field between the anode and cathode can be utilized to drive water desalination. For this application, the conventional ion exchange membrane is replaced by a thin chamber, sandwiched by an anion exchange

membrane (AEM) and a cation exchange membrane (CEM). Upon operation, the inner electrical field drives the anions (e.g., Cl^-) and cations (e.g., Na^+ and Ca^{2+}) to migrate through the AEM and CEM towards the anode and cathode, respectively, thereby desalinating the salt water present in the thin chamber (Fig. 1d). Cao et al. [105] were the first to describe this novel approach. A high desalination efficiency of 90% was achieved in a single cycle, outperforming traditional, energy-intensive water desalination technology.

The energy source of MEs systems comes from microbial metabolism at the anode. Limited by the activity and conversion efficiency of these microorganisms, MESs are more suitable for treatment of domestic wastewater than for treatment of industrial wastewater that contains heavy metal ions and chemical toxicants. In order to meet the strict environmental standards for domestic wastewater treatment, the concentration of organic components and nutrients like nitrogen and phosphorus need to be reduced. Numerous studies have shown excellent MEs performance in decreasing organic components (99% acetate removal was achieved in [106]), but the removal of nutrients requires aerobic conditions not compatible with the anaerobic conditions at the anode [86]. Wang et al. [107] developed a novel electrochemical membrane bioreactor, in which wastewater was treated by the microorganisms at the anode and then filtered at the cathode, releasing a final effluent of high quality. This study highlighted the possibility of simultaneous wastewater treatment and net energy production in an MES. Kelly and He [86] reviewed the influencing factors and challenges for nutrient removal and recovery in various MESs including MFCs and MECs. In addition, Nam et al. [108] demonstrated that a high nutrient concentration can negatively affect power generation by MFCs; specifically, an initial concentration of TAN (total ammonia nitrogen) over 500 mg N L^{-1} strongly decreased electricity production in MFCs by inhibiting the anode-attached bacteria. The peak power density dropped by 59% when the initial TAN increased from 500 to 4000 mg N L^{-1}. For simultaneous COD and nutrient removal, configuration innovation and system integration are needed. Gao et al. [109] proposed an integrated system that combined an MFC and an electric membrane bioreactor. In this configuration, the effluent from the MFC was driven to flow through an air-contact oxidation bed and trickling filter to enhance TAN removal by leveraging the aeration effect. The efficiency of both COD and TAN removal exceeded 93% in this system.

Although significant strides have been made in the development of MESs, their performance (in terms of power density) is still lower than the industry standard of 1 kW m^{-3}. Exceptions to this do exist in several microscale bioreactors that have achieved industry-level values for power density [86, 94]. Factors that have potential for improvement include the following: (i) biomass and microorganism catalytic activity; (ii) mass transport between bulk and reactive region; (iii) electron transport between microorganisms and solid electrodes; and (iv) material conductivity and durability.

4.2 Bioreactors in Microbial Electrochemical Systems: The Fundamentals

MESs employ the electrochemical activity of certain microorganisms that oxidize organic or inorganic (e.g., sulfides) substrates to produce electrons during their anaerobic respiration. These electrons are transferred to solid electrodes either directly via membrane-bound protein structures such as nanowire and c-type cytochrome, or indirectly using mobile redox electron shuttles [85]. As a result, a negative anode potential of about −0.2 V versus SHE (Standard Hydrogen Electrode) is obtained. Meanwhile, due to the sluggish reaction kinetics with non-precious metal catalysts, the cathode potential can only reach about +0.3 V, together providing an open circuit voltage of +0.5 V [101]. The efficiencies of the reaction at the anode and of electron transport are the cornerstones of the MES, and they are currently the limiting factors of overall system performance [94]. It should be mentioned that an exhaustive electron transfer mechanism remains to be established, and the interactions between direct and indirect electron transfer methods still need further investigation. Both the anode and cathode reactions are usually conducted in different chambers of the bioreactor, at least one of which is catalyzed by microorganisms. Therefore, the bioreactor plays a significant role in the bio-electrochemical reaction rate and electricity production.

4.2.1 Configurations

As described above, a typical MES consists of two chambers: the anode chamber for electron production and the cathode chamber to close the circuit and yield the final products. MESs have evolved from typical two-chamber configurations to single-chamber and hybrid designs. Novel modes of operation like the up-flow mode have also been developed. In a two-chamber MES, aqueous and gaseous substrates are bio-electrochemically degraded to produce electrons in the anode. These electrons are transferred to the cathode, resulting in electricity production or product generation. The first single-chamber MFC was described by Liu et al. [110] as shown in Fig. 3a. They demonstrated that atmospheric oxygen can passively diffuse into and react with the porous hydrophobic cathode. Plain anode and cathode can also be used to form a single-chamber MES bioreactor (Fig. 3b). Single-chamber MFCs are capable of treating wastewater with a high concentration of nitrogen [106], although ammonia inhibition was still observed. The maximum power density decreased from 6.1 to 1.4 W/m^3 when TAN concentration increased from 3500 to 10,000 mg/L. One concern for the single-chamber MFC is that a large percentage of the organic substrate is lost without contributing to electricity production [110].

From a geometric perspective, both the single- and double-chamber MESs can be engineered to form a tubular configuration. This configuration is considered very promising due to increased sludge retention time and reduced hydraulic retention

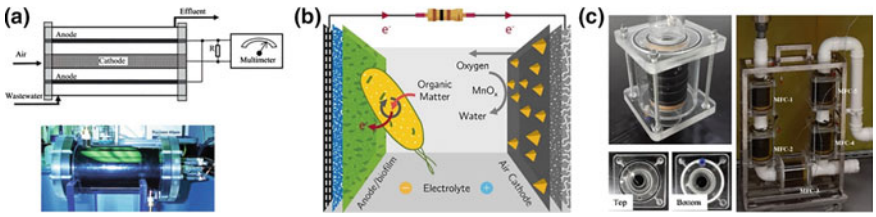

Fig. 3 Configurations of microbial electrochemical systems (MESs). **a** Schematic and prototype of the first single-chamber MFC (adapted and reprinted from [110], Copyright 2004, with permission from American Chemical Society), **b** Schematic illustration of a single-chamber MFC with plain anode and cathode (adapted and reprinted from [111], Copyright 2016, with permission from Elsevier), **c** Tubular two-chamber MFC, and 5 MFCs were connected to form a stack and integrated into a sink drain pipe (adapted and reprinted from [112], Copyright 2016, with permission from Springer)

time. More importantly, tubular MESs can be readily integrated to fit into existing facilities [100]. Rabaey et al. [113] proposed a tubular single-chamber MFC using packed granular graphite as the anode and a woven graphite mat as the cathode. Ye et al. [112] developed tubular two-chambered MFCs using PMMA (Polymethyl Methacrylate) tubes of different diameters; the inner tube and the interspace served as the anodic and cathodic chambers, respectively. Five of these MFCs were integrated into a sink drainpipe for kitchen wastewater treatment (Fig. 3c). The flushing process was found to disturb the performance of the MFC, but only for a few minutes. On the contrary, an irreversible drop in MFC performance was observed after flushing the substrate at 50 °C. Conventional tubular two-chamber MFCs usually have a concentric cylinder configuration (Fig. 3c), where a cation exchange membrane covers the inner cylinder to segregate the anode and cathode chambers and to allow proton transport. However, many other configurations have also been developed. Li et al. [114] proposed a single-chamber MFC with a cathode on either side of the anode chamber. Their results showed that the volumetric power density was positively correlated with the ratio between cathode surface area and anode volume, implying that a larger cathode surface area can lead to better performance.

Recently, the unique advantages of miniaturized platforms (i.e., microfluidics and lab-on-a-chip devices) in microbial research have been recognized. These methods provide better microenvironment manipulation for cell and biofilm culturing, as well as high-throughput and time-effective approaches for characterization [89, 115–118]. In addition, by leveraging advanced microscopy technologies as a powerful research tool, microfluidic chips enable real-time and in situ imaging even at a single-cell level [119]. Qian et al. [120] developed a two-chamber MFC with an anode chamber volume of 1.5 μL to investigate microbe-anode interaction, and succeeded in enhancing biofilm growth on the anode electrode. Qian et al. [121] also developed a PDMS (Polydimethylsiloxane)-fabricated MFC with a chamber volume of 4 μL. This MFC showed faster start-up and higher power density. Their results suggested the volumetric power density was inversely

correlated with chamber volume. However, one major challenge that still hinders the application of miniaturized platforms is the clogging that results from the growth and aggregation of microorganisms over time [122].

4.2.2 System Integration

The output of a single bioreactor is usually insufficient for most applications. One promising approach to this problem is to combine several bioreactors to form a stack, which improves productivity and efficiency. For example, several MFCs can be hydraulically and electrically connected to form an MFC stack. This approach does not affect the coulombic efficiency of individual fuel cells but can increase the total power output and COD removal efficiency [123]. Ledezma et al. [124] demonstrated the first self-sustainable MFC stack that is not only self-sufficient (in terms of feeding, hydration, sensing, and reporting), but can also produce sufficient net power output to run peristaltic pumps. Research has revealed that MFC configuration, as well as the hydraulic and electric connections in stacked MFCs, have to be properly engineered to avoid short-circuiting and to fulfill the requirements of the desired application. One major challenge for MFC stacks is voltage reversal (where one or more MFCs reverse polarity), which results in severe deterioration of the MFC system as a whole. Oh and Logan [125] investigated voltage reversal in two air-cathode MFCs connected in series. They found that the MFC voltage can reverse under conditions of low fuel or in the absence of bacterial activity. They suggested the development of a control strategy that isolates the reversed MFC while still maintaining power output from the remainder of the stack. In order to avoid voltage reversal of any one fuel cell, all the cells in the MFC stack need to have exceptionally consistent performance characteristics. This is quite challenging in practice, especially when the cells are hydraulically connected in series. Alternatively, electrical engineering approaches can be utilized to mitigate voltage reversal. For instance, capacitors can be integrated into a serially connected MFC stack to accumulate charge, which would prevent voltage reversal and enhance power output [126, 127]. Kim et al. [127] integrated an MFC stack with two sets of multiple capacitors, which were alternately charged and discharged at a frequency of 1 Hz. The capacitors were charged in parallel to avoid voltage reversal of the MFC stack but were discharged in series to increase the voltage output ($\sim$2.5 V). Wu et al. [128] developed a DC/DC booster circuit to increase the voltage of an MFC stack to more than 3 V. However, one commonly ignored issue is that an MFC stack is merely a composite of single or double-chamber MFCs, and therefore suffers from the same disadvantages as individual cells. For example, single-chamber MFCs suffer from decreased coulombic efficiency [129]. Double-chamber MFCs suffer from anode chamber acidification, which is amplified when multiple two-chamber MFCs are hydraulically connected in series due to the gradual accumulation of protons in downstream cells [130, 131]. Yang et al. [132] found that the aerobic oxidation of acetate by the microbial biofilm at the cathode of the single-chamber MFC was able to remove accumulated H^+ in the medium. Therefore, they proposed a hybrid MFC stack that combined single- and

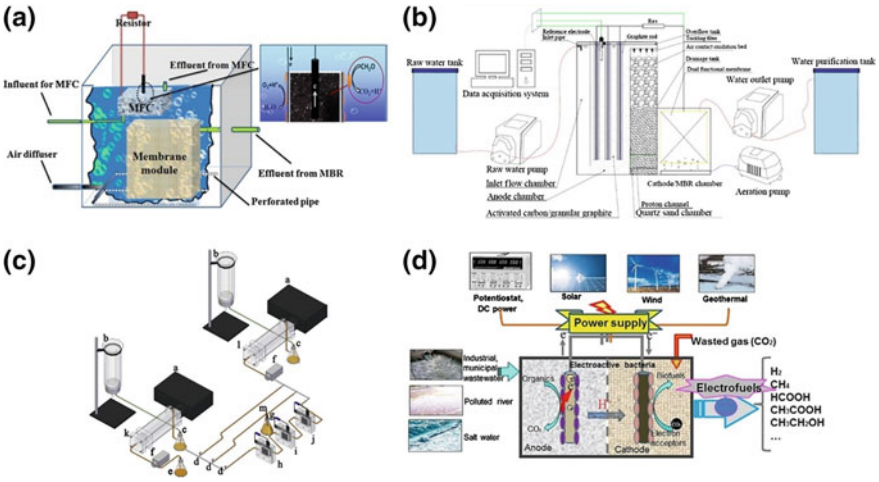

Fig. 4 Schematic illustration of typical integrated MESs. **a** An integrated MFC-membrane bioreactor (MBR) system. The oxygen in the MBR aeration tank was further utilized by MFC for electricity generation and more efficient treatment (adapted and reprinted from [134], Copyright 2012, with permission from Elsevier), **b** a combined system with MFC and electric membrane bioreactor (EMBR). This system exploited the hydraulic pressure difference between the anode and cathode chambers to drive the MFC effluent to flow through an air contact oxidation bed and trickling filter for enhanced nitrogen removal (adapted and reprinted from [109], Copyright 2017, with permission from Elsevier), **c** a combined MFC and photosynthetic biohydrogen reactor (PBR) system. The effluent from upstream PBR was treated by MFCs to remove inhibitory byproducts and protons, enhancing the performance of downstream PBR (adapted and reprinted from [135], Copyright 2013, with permission from Elsevier), **d** MESs can also be integrated with renewable energy systems to maximize the energy efficiency (adapted and reprinted from [136], Copyright 2017, with permission from Elsevier)

double-chamber MFCs to achieve self-sustaining pH control and avoid anodic acidification.

Bioreactors do not serve as stand-alone devices; they need to be integrated with other MESs and even other energy systems for maximum performance and energy efficiency. Liu et al. [133] proposed an integrated MFC-SBR (sequencing batch reactor) for the activated sludge process. The MFC was submerged into the SBR, synthetic wastewater was fed to the MFC first, and the resulting effluent was processed by the SBR. The oxygen for the aeration process was shared by the MFC biocathode to further recover electrical energy and reduce the cost of operation. Wang et al. [134] integrated an MFC and a membrane bioreactor (MBR) to develop a hybrid system (Fig. 4a) where the excess oxygen in the aeration chamber of the MBR was used to enhance the cathode performance of the MFC and the electricity produced by the MFC partially offset the overall energy cost. Gao et al. [109] proposed an integrated system with an MFC and an electric MBR. The conventional proton exchange membrane was replaced with a quartz sand chamber (Fig. 4b). The effluent from the MFC was run through an air-contact oxidation bed

and trickling filter to exploit the aeration reaction for nitrogen removal. This hybrid system achieved >93% efficiency in COD and TAN removal, and 50% efficiency in phosphorus removal. Li et al. [135] integrated two photosynthetic biohydrogen reactors (PBR) with three MFCs to form a PBR1-MFCs-PBR2 system to produce additional hydrogen from PBR2 (Fig. 4c). The idea was to utilize the MFC to degrade soluble intermediate products (e.g., lactate, propionate, and butyrate) generated from PBR1 thereby (i) removing excess H^+ for pH adjustment and (ii) changing the volatile fatty acid composition to facilitate hydrogen production in downstream PBR2. The results showed that this configuration outperformed traditional series-connected PBR systems (PBR1-PBR2) by reaching a 15-fold increase in hydrogen production rate, and also surpassed conventional pH adjustment methods (PBR-pH regulation-PBR2) by achieving a fourfold improvement in hydrogen production rate.

MESs can also be coupled to renewable energy sources (e.g., solar, wind, and geothermal energy) to maximize the energy production of the entire system (Fig. 4e). These renewable energy sources are naturally intermittent and their power output is not as stable as conventional power plants, which leads to big fluctuations and potential risks for the electric grid [137–139]. Alternatively, the electricity produced by these renewable sources can be used to power MESs, within which reactions are mild and can easily be started or stopped. Before successful execution of this strategy, the electrical and material (e.g., substrate and oxygen) flux at both the bioreactor and system levels should be properly distributed and regulated for safe and efficient operation.

4.2.3 Influencing Factors

Since MES performance is dominated by bio-electrochemical reactions, it is expected that factors affecting reaction kinetics such as external resistance, operation mode, and environmental elements will have a significant influence on MES performance. Aelterman et al. [140] studied the electrochemical performance of MFCs with different three-dimensional electrodes. Their results showed that lowering the external resistance from 50 to 10.5 Ω increased the kinetic capacity of the microbes in the bioreactor and caused a threefold increase in electricity generation. The authors suggested that an MFC should be operated at an external resistance closely matching its internal resistance, in order to increase COD loading rate and subsequent electricity production. Zhang et al. [141] performed a detailed investigation of the effect of external resistances on biofilm formation and electricity production in MFCs. When external resistance was decreased from 1000 to 50 Ω, the maximum power density increased by $\sim$181%. On the contrary, an even lower external resistance of 10 Ω caused a decrease in MFC performance due to low biomass activity and high extracellular polymer content in the biofilm. This study demonstrated that biofilm structure plays a crucial role in MFC performance.

Continuous and batch modes are the two typical modes of operation for MESs. In continuous mode, the substrate and catholyte are fed into the MES continuously,

whereas in batch mode, they are fed in all at once and then stored in the MES chamber for subsequent treatment. Although it is still unclear whether continuous mode outperforms batch mode in terms of performance and energy efficiency, the continuous mode of operation does have the advantage of being more productive, less labor-intensive, readily regulatable, and easier to control [96]. Kim et al. [106] compared the performance of a single-chamber MFC operated in either continuous or batch mode. They found that the MFC operated in continuous mode showed much higher TAN tolerance and sustained a 6.5-fold increase in TAN concentration when compared to the MFC in batch mode. However, a relatively long acclimation period of 40 days was required to achieve optimal electricity production in continuous mode.

Zhang et al. [142] investigated the effect of anolyte recirculation in MFCs with a floating air-cathode. Their work suggested that recirculation rate has a significant influence on proton transfer, fuel cell performance, and coulombic efficiency. Patil et al. [143] investigated the effect of temperature on biofilm formation and performance in MESs. They found that high temperatures not only accelerate biofilm formation but also increase biofilm activity. On the contrary, when the operating temperature was low, biofilms grown at low temperatures outperformed those grown at higher temperatures, implying the existence of a compensatory biological mechanism that accommodates environmental factors like temperature. The same study also found that the temperature limit for biofilm growth was approximately 0–50 °C, in which range the biofilm can adjust reversibly to temperature fluctuations.

4.3 The Importance of Bioreactors in Microbial Electrochemical Systems

4.3.1 Biomass Cultivation

The functionality of MESs relies on the anode reaction, which means that their performance is dominated by the metabolic activity of microorganisms [94]. A strong correlation between microbial metabolic rate and growth rate has been observed [96], implying that growth rate can be used as an indicator to predict biofilm performance. The stability of the microbial biofilm is dictated by substrate transport and the chemical signaling between multiple species [144]. Bhattacharjee et al. [144] fabricated surfaces with different topographies to regulate biofilm growth. Biofilm morphology was found to strongly correlate with the topography of the membrane material. Fluid flow is known to affect the three-dimensional morphology and bacterial communities forming the biofilm by generating shear stress and regulating molecular transport. Thomen et al. [145] developed a microfluidic platform that enabled hydrodynamic control and in situ observation of biofilm development. They determined the shear stress threshold for biofilm formation.

Hydrodynamic shear stress was found to regulate biofilm growth by controlling oxygen distribution.

Proton transport between the reaction solution and the biofilm also has a significant influence on the performance of the microbial biofilm. It has been shown that the protons accumulated inside the microbial biofilm can induce acidification, which severely inhibits biofilm metabolic activity [130]. Several methods have been proposed to mitigate this acidification, either by operating the bioreactor in continuous mode or by adding a buffer to regulate the pH of the surrounding milieu. Li et al. [146] proposed a new method to overcome acidification by periodically reversing the polarity of the MFC, thereby neutralizing the accumulated protons and hydroxyl groups. In addition, they showed that polarity reversal further enhanced the anode and cathode performance. Long-term stability (>4 months) with an ultra-low phosphate buffer concentration of 5 mM was also demonstrated. Yang et al. [132] proposed a hybrid MFC stack that utilized single-chamber MFCs to remove the accumulated H^+ produced by the double-chamber MFCs. Liao et al. [147] proposed to operate an MFC in alkaline media, as they found that the MFC operated with a repeating pH sequence (pH 7-8-9-8-7) achieved the highest performance. Electrochemical and biological analyses confirmed that the enhanced fuel cell performance was induced by the synergistic effects of highly active biomass and low internal resistance.

In addition to the reactions at the anode, the biomass in the cathode chamber can also contribute to enhancing reaction kinetics and producing bio-product. Park et al. [148] showed that the cathodic biofilm contributed to removal of organic components and nitrogen. Commault et al. [149] demonstrated that microalgae at the cathode can generate oxygen via photosynthesis to improve cathode reaction kinetics, resulting in a 100% increase in maximum power density. In addition, the cathodic microalgae also contributed to ammonium removal and algal biomass production. However, it was noted that the cathode biofilm can induce irreversible alkalization by inhibiting clearance of hydroxyls, also known as air-cathode biofouling [150]. Since cathode biofouling can be compensated by gradually increasing anode performance, it cannot be directly detected from the polarization curve [151]. Oliot et al. [151] developed a novel MFC enabling easy air-cathode replacement. Their results suggested that replacing the air-cathode can enhance fuel cell performance by 108% and 180% for anode areas of 9 and 50 cm^2, respectively. This study acknowledged the existence of biofouling and proposed a promising solution.

Significant effort was focused on increasing biomass production and enriching specific microorganisms that directly contribute to electron generation; however, little is known about the competition and evolution of microorganisms after inoculum [100]. As a result, one can only tune the system's performance using empirical correlations, which are quite apparent and facility-dependent. There is a long-standing debate over whether mixed microorganism cultures can outperform pure cultures. Increasing evidence points toward the mixed microorganism cultures, which show higher productivity and better tolerance to environmental impacts [152, 153]. Other benefits include substrate flexibility and less maintenance.

Conversely, mixed cultures normally involve several competitive bio-electrochemical processes and the electrosynthesis production efficiency exhibits considerable fluctuations [154, 155]. In order to target practical applications like the treatment of real waste materials (e.g., municipal and industrial wastewater, and biomass wastes), at least two types of microorganisms should be cultured in the MESs: one to break down complex polymers like cellulose and another to convert the resulting small molecules into electrons.

4.3.2 Mass Transport

Transport phenomena are crucial for MES performance, from the biofilm level (intracellular transport of biomolecules and extracellular transport of electrons/signal molecules) to the electrode level (substrate/product transport near the electrodes) and even at the bioreactor level (two-phase transport inside the anode and cathode chambers). In this section, we will focus mainly on the electrode and bioreactor levels.

As mentioned above, MES performance is not intrinsically limited by the microorganisms themselves, but by the biofilm microenvironment that is suboptimal for every bacterium. One can slightly enhance mass replenishment by increasing the reactant flow rate. However, this strategy is limited by uneven flow as well as increased power consumption. More importantly, the shear stress at high flow rates can destroy up to 50% of the microbial biomass and induce structural changes in the biofilm [156].

At the electrode level, the electrode configuration and material properties greatly affect biomass transport and enrichment. Conventional electrodes used in MESs were based on only graphite/carbon-based materials like graphite plate and carbon cloth. It has been demonstrated that biofilm distribution was not uniform and tilted towards the inlet end due to the continuous consumption of substrate and development of concentration boundary layer. Ye et al. [157] developed a microfluidic MFC and observed that biofilm thickness gradually decreased along the microchannel due to the severe diffusive mixing of the catholyte downstream. Compared to planar electrodes, the three-dimensional electrode can provide a larger surface area for microorganism attachment, and more importantly, can enhance mass transport by breaking the continuous development of concentration boundary layer. Cheng et al. [158] proposed a novel MFC in which the substrate was driven to penetrate a porous, carbon cloth-based anode in the through-plane direction. Although this approach could cause clogging, the maximum power density was improved by 17% as compared to the flow-over MFC configuration (substrate moving in plane with the anode). Recently, the unique advantages of three-dimensional electrodes (e.g., porous electrode, graphite rod array, and graphite granules) have been recognized. Jiang et al. [159] reported an MFC where the substrate was driven to flow through a 3D grapheme foam anode in the in-plane direction. Because of the convective mass transport and rapid replenishment of substrate inside the anode, this MFC yielded a volume power density of 745 $\mu W/cm^3$, which is higher than that of other devices.

Additionally, the consumption of culture medium and response time of this MFC were reduced by over 16-fold and fourfold, respectively, compared to non-flow-through devices. Graphite granules were also used to form a packed-bed anode for MFC operation. Rabaey et al. [160] found that packed-bed anodes made of granules can lead to a two-fold increase in MFC voltage, as compared to a plate anode. Additionally, fuel cell voltage can be further improved by inducing a cross-flow in the granular bed using baffles. However, other studies suggested that the graphite granules have a higher electrical resistance compared to graphite and carbon felt, and therefore decrease performance [140]. Recently, novel electrode materials with multi-scale porous structures were invented. Xie et al. [161] proposed a novel strategy to boost the effective anolyte-biofilm-anode reaction area by employing a unique, two-scale porous anode material made of carbon nanotube-textile (CNT-textile) composite. This two-scale porous structure featured (i) intertwined CNT-textile fibers that form a macroscale three-dimensional space for efficient substrate transport and microorganism colonization, and (ii) a microscale porous CNT-textile layer for enhanced electron transfer from biofilm to electrode. The MFC equipped with this CNT-textile composite outperformed the one with traditional carbon cloth; the maximum current density and power density were increased by 157% and 68%, respectively. In summary, an optimal electrode configuration for MESs should fulfill the following requirements: porous framework and biocompatible surface for microorganism attachment, large pore size for efficient mass transport inside the electrode, exceptional electrical conductivity, great corrosion resistance, convective replenishment for reaction depletion, and rapid product removal.

At the bioreactor level, several strategies to enhance mass transport have been reported. Li et al. [114] placed two baffles in the anode chamber to enhance the mixing of wastewater and active sludge (Fig. 5a). Jiang et al. [159] proposed a microfluidic MFC equipped with a flow-through porous anode (Fig. 5b). The interconnected pores of this graphene foam anode allowed convective enhancement of the electrochemical interactions between the microbes and the substrate. In addition, the unique scaffold structure of the graphene foam anode also enabled efficient diffusive nutrient transport to the biofilm. Compared with non-flow-through configurations, this flow-through MFC achieved 16-fold lower consumption of the culture medium and fourfold lower response time for electricity generation. Liao et al. [162] enhanced substrate transfer by rotating the carbon-brush anode (Fig. 5c). Compared with the non-rotating anode, the MFC with a rotating anode yielded 1.4 and 2.7 times higher peak power density and current density, respectively. It should be noted that external power was needed to drive the motor, which is not favorable for maximum system efficiency. Generally, buffers like phosphate or bicarbonate are added to the substrate to maintain the pH and facilitate proton transport to the cathode. Operating the system without buffering usually slows down proton transfer rate, thereby limiting the performance of the bioreactor. However, in practical applications, the use of buffers have to be minimized or eliminated, as they not only increase cost but also induce secondary

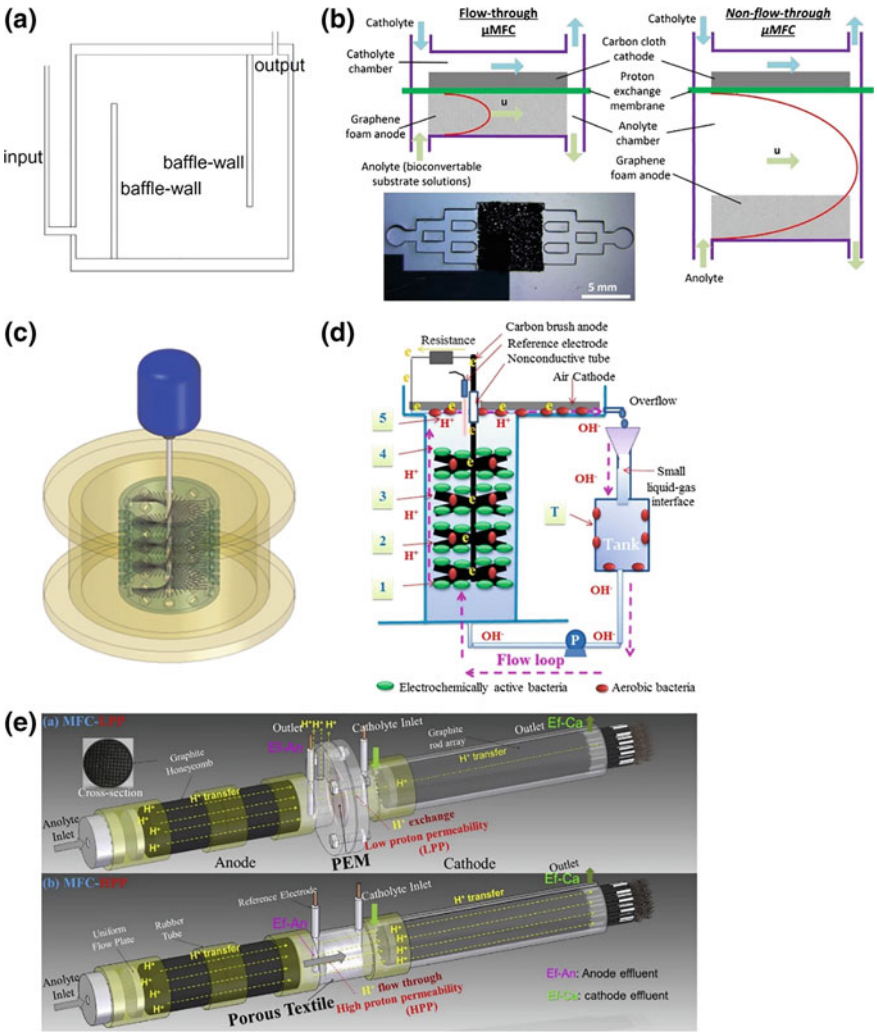

Fig. 5 Typical mass transport enhancement methods in the area of MESs. **a** Baffles were inserted in the anode chamber to enhance the mixing of wastewater and active sludge (adapted and reprinted from [114], Copyright 2008, with permission from Elsevier), **b** the substrate was driven to flow-through porous anode to convectively enhance mass transport (adapted and reprinted from [159], Copyright 2017, with permission from Springer), **c** a rotating brush anode was introduced to enhance substrate transfer (adapted and reprinted from [162], Copyright 2015, with permission from Elsevier), **d** The anolyte was recirculated to enhance the proton transport (adapted and reprinted from [142], Copyright 2008, with permission from Elsevier), **e** schematic of the continuous-flow tubular MFC with PEM and porous textile. The proton transfer and cell performance can be enhanced by flowing the anode effluent through the cathode electrodes (adapted and reprinted from [163], Copyright 2015, with permission from Elsevier)

contamination and/or eutrophication [163]. Zhang et al. [142] proposed a novel MFC with a floating air-cathode that allowed anolyte recirculation to enhance proton transport in buffer-free conditions (Fig. 5d), resulting in higher voltage output and higher coulombic efficiency. However, this recirculation strategy for effective proton transfer also causes excess oxygen transport into the anode, and the latter was found to deteriorate fuel cell performance at recirculation rates above 0.35 mL/min. In this study, the feasibility of enhancing the performance of unbuffered MFC via anolyte recirculation was also demonstrated. Zhang et al. [163] proposed another novel method to enhance proton transport by directly running the anode effluent through the cathode electrodes (Fig. 5e). Compared to membrane-segregated MFCs, this method improved the maximum power density by 125%. Further analysis indicated that the enhanced proton transport and increased catholyte conductivity contributed 51% and ~40%, respectively, to total performance improvement.

4.3.3 Energy Conversion

Energy conversion in MESs is a relatively complicated process that needs comprehensive analysis. For instance, the commonly reported performance metrics (i.e., cell voltage, current and power density, coulombic efficiency) only assess electrical energy, while ignoring chemical energy like methane [109, 123] and biomass production [149, 164–167]. He [88] suggested using metrics like energy density (kWh/m^3) or COD removal (kWh/kg) to properly estimate the energy conversion from organic substrate to electricity and better assess MFC performance. He also performed an energy balance analysis, which suggested that the electricity produced by MFCs hardly compensate for their power consumption in a wastewater treatment plant or generate net energy output at the system level. Following this analysis, the major promotion for the implementation of MFC in the wastewater treatment process should be reduced power consumption and less sludge production, as compared to conventional aeration treatment.

Besides electricity, MESs also hold great potential in the production of value-added chemicals and biomass. Yu et al. [90] utilized MFCs and MECs to treat wastewater from a high-strength soybean edible oil refinery (SEOR) while simultaneously producing electricity and methane. The methane was produced at an efficiency of 45.4 ± 1.1 L/kg-COD and a rate of 0.133 ± 0.005 $m^3/(m^3\ d)$, which was higher than that obtained in non-electrochemical anaerobic digestion. Zhou et al. [167] integrated an algal biocathode with a dual-chamber MFC to provide oxygen to the MFC cathode via the photosynthetic activity of the algae. The CO_2 produced at the anode was further converted to biomass at the cathode, enabling simultaneous wastewater treatment, electricity generation, and biomass production. Commault et al. [149] developed an MFC equipped with a photo-cathode, in which the wastewater was pretreated by anodic bacteria and then further treated by cathodic microalgae to produce electricity and algal biomass. Ma et al. [164] reported a photosynthetic MFC that also used microalgae-mediated oxygen

production to enhance the oxygen reduction reaction at the cathode. The energy flow analysis of this system suggested that the production of algal biomass took a majority of the recovered energy, and the net electricity production did not meet expectations.

Another issue regarding energy conversion by different types of MESs is the metric used for quantification and comparison. Current density based on the projected surface area is the most commonly reported parameter. However, the widely used porous electrode materials usually have a much larger inner surface area than the projected surface area, causing the current density calculation to overestimate system performance. Sharma et al. [168] published a critical review about the key parameters for assessing MESs, and provided guidelines to correct current and exchange current densities based on different surface areas (e.g., biofilm covered area, electrochemically active surface area) of the electrodes. In addition, the authors also suggested including the robustness of electrochemically active biofilms as a performance indicator.

5 Conclusions

Microbial energy conversion technology is a promising approach to relieve the burden on fossil fuels and decrease environmental pollution. Bioreactors play a very important role in microbial biomass production and energy conversion. This chapter presents a fundamental understanding of the functions, configurations and influencing factors of bioreactors with respect to their application in microbial biomass cultivation, microbial biofuel conversion, and microbial electrochemical systems. Bioreactors can provide a suitable and stable place for microbial growth and metabolism by appropriately controlling their operating conditions. In addition to the operating conditions, the performance of a bioreactor is greatly influenced by many other factors like structure and size, mixing and transfer characteristics, means of feed introduction and product removal. In particular, bioreactors exhibit complex multiphase flow patterns that result in varying heat and mass transfer characteristics in microbial biomass and energy conversion. However, the mass and heat transfer efficiency in bioreactors is low, leading to a poor biomass and biofuel productivity. The economic viability and competitiveness of microbial energy conversion in bioreactors are much lower than that of petroleum-based energy sources. As a result, the energy conversion efficiencies of bioreactors are poor and unstable. Further optimization of bioreactor structure and operating conditions, mass and heat transfer, as well as reactant activity should be conducted.

In conclusion, bioreactor-based microbial energy conversion is a very promising technology and holds great potential for commercialization as a sustainable energy source. Bioreactor development and system integration are already underway, and several field tests have been reported. However, further studies to improve performance, scalability, and reliability are needed to make bioreactor-based microbial energy conversion a commercially applicable energy source.

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