Renewable and Sustainable Energy Reviews 101 (2019) 548–558

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

Intervention of microfluidics in biofuel and bioenergy sectors: Technological considerations and future prospects

Rintu Banerjee[a,1], S.P. Jeevan Kumar[a,e,1], Ninad Mehendale[b], Surajbhan Sevda[c], Vijay Kumar Garlapati[d,*]

[a] Agricultural & Food Engineering Department, Indian Institute of Technology Kharagpur, Kharagpur 721302, West Bengal, India
[b] Ninad's Research Lab, Thane 400602, Maharashtra, India
[c] Department of Biosciences & Bioengineering, IIT Guwahati, Guwahati 781039, Assam, India
[d] Dept. of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Wakhanaghat 173234, Himachal Pradesh, India
[e] ICAR-Indian Institute of Seed Science, Mau 275103, Uttar Pradesh, India

ARTICLE INFO

Keywords:
Microfluidics
Biofuels and bioenergy
Fabrication
Bioreactor
Microalgal biodiesel
Microbial fuel cell

ABSTRACT

Biofuels/Bioenergy is renewable in nature by mitigating the greenhouse gas emissions despite rapid economic growth and energy demand. Biodiesel and bioethanol production from renewable sources are gaining much attention but unable to translate the technologies into commercially ventures. Several technical challenges like the screening of algae/yeast for higher lipid accumulation/ethanol production, separation and purification of microalgae from contaminants, harvesting of microalgae, improving transesterification efficiency with meager solvent consumption, energy and time have been addressed using microfluidic devices. Besides, it has shown promising results in microbial fuel cell domain. Microfluidics and microreactors offer miniaturization of experiments by a very little expense of solvents, energy and time with higher precision results. Moreover, it provides 19.2% higher surface to volume ratio when compared with Petri dish (35 mm diameter) and microchannel (50 μm tall, 50 μm wide, and 30 mm long). Higher surface to volume ratio is helpful in the integration of the whole laboratory (i.e., lab-on-a-chip), where efficient screening of ethanol/lipid producer, higher transesterification efficiency could be ascertained. Due to the overwhelming potential of microfluidics in biofuel and bioenergy sectors, the present review article illustrated several examples to depict the importance of microfluidics towards high-throughput analysis of screening the potent microbial/microalgal strain, fabrication of microfluidic bioreactor, quality analysis of biofuel and bioenergy products.

1. Introduction

Biofuels and bioenergy are gaining significant thrust owing to the greenhouse gas reduction potential despite fast economic growth, dwindling of natural resources and burgeoning population [1]. Biofuels, particularly biodiesel and bioethanol are renewable, non-toxic and promising liquid fuels with a potential to reduce greenhouse gas emissions [2]. Besides, recent investigations emphasize the importance of microbial fuel cells as one of the prominent energy sources that can satiate the energy demand.

Biodiesel is one of the renewable fuel, is produced from edible and non-edible oils, waste oils, animal fats and single cell oils (algae, fungi, yeast and bacteria) [3,4]. Research on vegetable oils (soybean oil in USA, mustard and sunflower oil in Europe and palm oil in Malaysia and Indonesia) and non-edible oils (Jatropha, Karanja) have been extensively studied to be deemed for alternative fuel; however, streamlining food commodities into fuel invited food vs fuel dilemma, requirement of land acreage, exorbitant cost and poor yield are some of the reasons to defeat the purpose of using these oils for biodiesel production. Recent studies substantiate the enormous potential of single cell oils (microbial lipids) because of broad-spectrum utilization of lignocellulosics, glycerol and waste sources for higher lipid

Abbreviations: ASTM, American Society for Testing and Materials; DI water, Deionized water; EN, European; FAEE, Fatty Acid Ethyl Ester; FAME, Fatty Acid Methyl Ester; KOH, Potassium Hydroxide; MEC, Microbial Electro Chemical; MEMS, MicroElectroMechanical systems; MFC, Microbial Fuel Cell; MMFC, Microfluidics-based MFC; $NaBH_4$, Sodium BoroHydride; NIR, Near-infrared; NMR, Nuclear Magnetic Resonance; PDMS, PolyDiMethylSiloxane; RTD, Resistance Temperature Detectors; SAV, Surface Area to Volume

* Corresponding author.
E-mail address: garlapati.vijaykumar@juit.ac.in (V.K. Garlapati).
[1] Equal contribution as first author.

https://doi.org/10.1016/j.rser.2018.11.040
Received 8 June 2018; Received in revised form 21 November 2018; Accepted 26 November 2018

accumulations [3]. Besides, these single cell oils can be easily scaled-up with short incubation time and uncompetitive with the food chain. Single cell oils (microbial lipids) production comprises several important steps such as cultivation of strain, biomass harvesting, lipid extraction, transesterification and purification of fatty acid methyl esters (FAME). Among these steps, screening and selection of strain, optimization of influential parameters for higher lipid accumulation, consumption of less solvent and energy are viable options for a commercially biodiesel production process [5]. On the other hand, microbial fuel cell technology and bioethanol are also promising bioenergy options due to their potential for reduction of greenhouse gas emissions [6,7]. Bioethanol production consists of delignification, saccharification and fermentation steps, where screening of both isolated and genetically engineered *S. cerevisiae* potential for ethanol fermentation is one of the prominent steps for higher ethanol production. These screening steps are exorbitant, requires energy and time that makes the whole process extortionate. Besides, microbial fuel cells is a viable technology for energy production but suffer from the integration of various components, which culminated the whole process exorbitant. Microfluidics is one of the viable technology to deal with the existing barriers with the biodiesel, bioethanol and microbial fuel cell technologies. The positive attributes of microfluidics include usage of smaller reagent volumes, lesser reaction times, miniaturization and ability to integrate the whole laboratory onto a single chip (i.e., lab-on-chip) [8,9].

Microfluidics is defined as a multidisciplinary science that deals with the handling and analyzing of fluids at the microscale [10]. Role of microfluidics in the screening of strain, optimization of variables for enhanced lipid/oil conditions, media engineering towards higher microbial lipid production have been attempted. These techniques have not only proved in enhancing the lipid/biodiesel production but also reduced the solvents usage, time and energy. Moreover, these techniques are more precise and help in accurate measurements particularly in determining the quality of blended biodiesel. Indeed, this field of interdisciplinary science has tremendous potential in bioenergy applications. To understand the microfluidics and their applications, a brief understanding of the fluid properties at microscale is necessary [11]. Hence, in the present review, we have put forth the hierarchy of microfluidics and its intervention in biofuel and bioenergy sectors along with the probable future insights towards a sustainable and cleaner world.

2. Features of microfluidics

Fluids behavior at microscale is distinctive than the existing macroscale systems. Microfluidic technology has high surface-to-volume ratio range from 10^4 (for channel dimensions in few hundred microns) to as high as 10^8 (for channel dimensions of few microns). The governing physical forces are different and can be characterized by low Reynolds number (Typically «1 but can go up to 100 in case of inertial microfluidic devices) [1], high Peclet number, high surface to volume ratio and slower diffusion.

2.1. Reynolds number

Reynolds number (Re) is an indicator of fluid flow that determines the nature of the fluid. In fluid mechanics, there exist two kinds of flow: laminar and turbulent (Fig. 1). In laminar flow, the particle's velocity in the fluid system is not random of time, which implies the particles in the two or more fluid systems cannot mix except by diffusion [12]. The prediction of flow can be indicated with Reynolds number, where the Re is < 2300 then it is laminar. Unlike laminar flow, turbulent flow is unpredictable and chaotic, where the position of the particle in the fluid is unpredictable as a function of time and characterized with Re > 2300. Being a laminar flow in microfluidics, it enables the researcher to study the predictable transport of stressor through micro channels, performing of assays, sorting of particles based on size and cellular analysis due to packets formation [13].

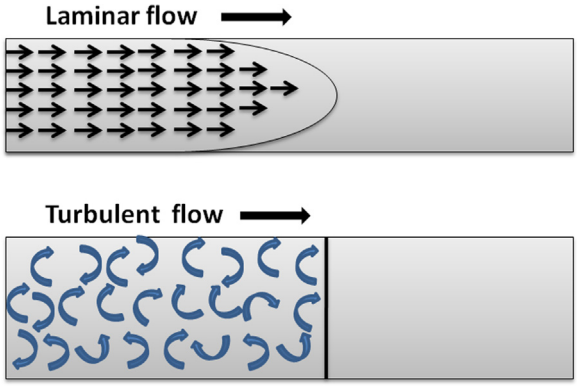

Fig. 1. Schematic representation of laminar and turbulent flow properties in microfluidics.

$$\mathrm{Re} = \frac{\rho v D_h}{\mu}$$

where ρ is the density of fluid, v pertains to fluid characteristic velocity, $D_h$denotes the diameter of hydraulic diameter and μ signifies the fluid viscosity. The hydraulic diameter (D_h) is a computed value, which relies on the cross-sectional geometry of a channel.

2.2. Peclet number

A Peclet number is a dimensionless number (ratio) that signifies the rate of advective transport to the diffusion transport. Concentration gradients occur either in gas mixture component or mass transport of dissolved solutes/particles. The high peclet number is observed in microfluidics, which enables the parallel flow of fluids in more extended fashion without mixing [14].

2.3. Diffusion

Diffusion is a physical process that is involved in the exchange of solutes/gases with Brownian movement depending on the concentration gradient. As the flow is laminar in microfluidics, the diffusion in one dimension is given as d2 = 2Dt, where D is the diffusion coefficient and d indicates the distance of a particle that moves in time t. As the distance differs from square power, the time of diffusion could be low at the microscale [15,16]. This property of the slow distribution of solutes/particles/gases could be exploited in creating the concentration gradients within microchannels [17,18].

2.4. Surface tension

In macroscopic scale, gravity and pressures play an important role in fluid dynamics; however, the capillary forces and surface tension remains negligible. On the other hand, at microscale level, the surface tension and capillary forces are significant in the fluids. Surface tension results based on the cohesive energy of particles that remain at liquid/gas interface. The free energy surface is an indicator of the tension prevailing on its surface [19]. In biodiesel synthesis process, surface tension has the vital role in lipid extraction, transesterification, and fuel atomization.

2.5. Surface to volume ratio

Surface to volume ratio (SAV) in microfluidics is always low and have the advantage of quick diffusion of particles/solutes/gases. This property is fundamental in transesterification reaction when the solvents are mixed co-axially; the SAV was higher on the methanol reagent

as a result mass transfer enhanced between the interfaces of oil/methanol [20]. This effectively reduced the broad requirement of solvent to favor forward reaction (fatty acid methyl esters formation). Also, lipid extractions from biomass also can be enhanced due to improved penetration of solvents for efficient lipid extraction [21]. Generally, it is not uncommon to observe increase of surface area to volume (SAV), when shifted from macroscale to microscale. A petri dish half filled with water having 3.5 mm diameter, 2.5 mL volume possess a ratio of 4.2 cm^{-1}; whereas, a microchannels with height 50 m, wide 50 m, long 30 mm, a 75 nL volume have a SAV ratio of 800 cm^{-1}. This property will have wide applications in transesterification reaction that reduce the volume of solvent consumption and energy requirement. Besides these physical forces, capillary forces and interfacial tension are significant in microfluidics than the gravity. These properties are responsible for the unique nature of fluid behavior and can be used for downstream processing, lower cost, faster results and better control [10,22].

3. Applications of microfluidics in biodiesel production

3.1. Microfluidics for process conditions towards microalgal lipid production (light effect on lipid and growth)

Microbial lipids serve as an effective substrate for biodiesel production. Lipids in the oleaginous microbes accumulate under stress conditions such as nutrient and culture condition limitations (pH, light, C/N ratio etc)). Optimization of physical and nutrient requirements is necessary for enhanced lipid production. Conventionally, this process takes much time and cumbersome. Shih et al. have developed a digital microfluidic (DMF) device that has studied the role of light in enhancing the lipid content of *Cyclotella cryptica* [23]. An interesting phenomenon was observed that under yellow light (~580 nm) synthesis of more energy-rich lipids occurred. However, the yellow wavelength is not congenial growth. Hence, to balance the growth as well as lipid accumulation, the researchers had exposed the *C.cryptica* to alternative blue light for 15 h and yellow light nine h, respectively. This indicates under the blue light, *C.cryptica* has rapidly proliferated; whereas, yellow light (stressor) subjection lead to higher neutral lipid synthesis. Besides this standardization of light phenomenon, microfluidics has been applied in studying the role of nutrient limitations for lipid synthesis and physical factors [24]. Digital microfluidics is an advanced microfluidic technique that avoids the use of connectors. In this study, reservoirs for inoculums maintenance and dyes for lipid identification along with vents are provided.

3.2. Microfluidic device applications in microalgae-based biodiesel production

Emerging evidence emphatically substantiate the potential of algal lipids as an important alternative to the vegetable oils because of carbon sequestration ability and easy to scale-up. However, blockades such as impurity of cultures and harvesting are some of the major issues to be addressed. To ensure higher lipid productivity, culture purification of microalgae has to be maintained to make free from contaminants, as the latter competes with nutrients that ultimately hampers the biomass productivity, composition and quality [25]. *Tetraselmis suecica* is one of the prominent algae that have a potential for higher lipid production but vulnerable to contamination with *Phaeodactylum tricornutum* a diatom that outpaced Tetraselmisspor sp., Dunaliella sp. in co-culture particularly at extreme pH [26]. Syed et al. [27] had reported a low-cost spiral microchannel microfluidic device to separate and purify *Tetraselmissuecica* from *Phaeodactylum-tricornutum*contamination. Based on the study, they have found that with the optimum flow rate,95% of the *P. tricornutum* cells were separated without affecting the cell viability. This study corroborates the potential of inertial microfluidics to sort the desired strain from the contaminants and ensure higher lipid productivity [27].

In the algae-based biodiesel process, cultivation, harvesting, dewatering, oil extraction and transesterification are the major enumerated steps [5]. Microalgae, by tiny cell size and high diluted cultures (0.5–5 g L^{-1}), harvesting is a cumbersome and tedious process [28]. Hence, the development of cost-effective algae harvesting, where, huge water is to be removed is a major challenge in algal- based biodiesel process. Harvesting methods such as centrifugation, flocculation and ultrasound- based physical methods have been employed. However, cost of equipment, poor product recovery and hostile to scale-up the process are the major drawbacks with the conventional process [29,30]. To circumvent the problem, Barros et al., have proposed a two-pronged strategy, which comprises of thickening (pre-concentration) and de-watering steps [28]. Hønsvall et al., has developed a trilobite structure based microfluidic chip having a dimension of 5-μm gap limit and applied to concentrate the *Chaetoceros* sp, *Rhodomonas baltica* and *Thalassiosira weissflogii*, respectively. Interestingly, the chip had concentrated the rigid cells and sorted-out according to size. This property could envisage harvesting the algae ready for harvesting; whereas smaller size algae could be re-circulated to the reactor. Besides, this process avoids the requirement of expensive instruments like a centrifuge and remain a viable technology for the harvesting of algae [31].

3.3. Microfluidic device for transesterification study

Biodiesel can be produced by pyrolysis, emulsions, transesterifcation and mixing. However, the most common technique used for biodiesel production is transesterifcation because of low cost, lesser time and energy requirement (Table 1) [32]. Transesterification is an important reaction that determines the quality of biodiesel. In this reaction, the fatty acids react with methanol in the presence of the catalyst (acid, base, and enzyme) to form fatty acid methyl esters and glycerol. The main event in this reaction is the immiscibility of oil/lipid and methanol and excess methanol uptake to favor the forward reaction. To enhance the heat/mass transfer microfluidics techniques have great potential in accomplishing the task [33,34]. For instance, Yeh et al. [20] have developed a microdroplet microfluidic platform with large surface to volume ratio that drives the chemical reaction forward by increasing the material interface. In this method, soybean oil and methanol were used for transesterifcation. Methanol when passed co-axially to the pool of soybean oil, the former was surrounded by the latter and decreased the methanol to oil ratio (3:1). Moreover, oil conversion oil conversion occurred in 9 min with 80% at 23 °C. Interestingly, at 1, 1/2, and 1/3 methanol to oil ratios the oil conversions showed 100%, 99.5%, and 98.6%, respectively [20] (Fig. 2). This observation substantiates that the methanol reactivity could be enhanced with oil/lipids by increasing the material interface between the reagents and also reduce the cost and energy. Oil to methanol ratio is one of the important characteristic that determines the efficiency of transesterification. Kumar and Banerjee, has reported 1:15 CE 90:1 oil to methanol ratio for enzymatic and acid transesterification of *Trichosporon shonodae* and *Aspergillus splipids* into FAME conversion, respectively. Kumari et al., have had studied enzymatic transesterification with 1:15 alcohol to oil ratio because higher the alcohol ratio to oil, higher the yield of FAME. Unlike these conventional methods, microfluidics techniques could reduce the solvent consumption, energy and time [4]. Further, recent literature emphasized the utilization of green solvents in the transesterification [5] and harnessed such solvents with the fabrication of microfluidic devices could bolster the biorefinery products and ultimately deliver a viable and cost-effective process [35–38].

3.4. Microfluidic device for integration and in situ monitoring of biodiesel production

Transesterification reaction for biodiesel production is mainly carried out in stirred tank reactors; which had confronted with several

Table 1
Evaluation of biodiesel producing techniques their features and disadvantages [32].

Parameter	Direct use/blending	Microemulsions	Transesterification	Pyrolysis
Features	Easy and simpler technique to reduce biodiesel viscosity.	Microemulsion is a colloidal equilibrium dispersion of isotropic fluid microstructures formed from two immiscible liquids	A process to reduce viscosity by reaction of oil or fat with an alcohol to form esters and glycerol	Process of conversion a substance into other by heat or heat with catalyst is known as pyrolysis
Catalyst	Not required.	Not required	Required	Required
Time	Requires less time in comparison to other process	Requires longer time for stable emulsion	Time depends on mode of transesterification. Acid and enzymatic transesterification takes time, whereas in base transesterification completes reaction within an hour	Takes longer time
Energy	Very meager energy required.	Very meager energy required.	Energy required.	Operates at high temperatures and pressure and requires high energy
Cost	Expensive due to vegetable oils usage	Expensive	Inexpensive	Expensive
Drawbacks	Trumpet and coking formation in injectors Heavy carbon deposits and oil sticking Lubricating oil thickening and gelling due to vegetable oil contamination	Although viscosity reduced than oils but heavy carbon deposit, incomplete combustion and injector needle sticking are major hurdles	Separation of pure esters is not easy due to presence of mono and diglycerides in the esters. Causes turbidity due to monoglycerides in the mixture of esters	Uncontrollable reactions due to variety of reaction pathways.

problems like biphasic reaction, requires more steps (neutralization of base with acids and treatment steps intoxicate wastewater) to meet EN 1421419 [39] and ASTM 6751–1118 [40] standards and thermodynamic equilibrium [41,42]. To address these issues of batch reaction, a continuous reactor has been prepared using microreactors with fabricated channels of different shapes like omega-, Tesla-, and T-shaped coupled with a monitoring system for enhanced biodiesel yields (Fig. 3). From the results, it has been noticed that FAEE's have been increased with increase in ethanol concentration. Higher FAEE's were obtained with a residence time of 10 min in Tesla (96.7%), omega (95.3%) and T- shaped (93.5%) channels by utilizing 1% NaOH, 25:1 ethanol to oil molar ratio at 50 °C [43] (Fig. 4). The higher FAEE production is mainly attributed due to enhanced mixture efficiency. Further, to monitor the FAEE production a NIR spectroscopy has been coupled with the continuous fabricated microreactors. Unlike other chromatographic techniques, this technique is inexpensive, non-invasive and requires minimal sample. This technique demonstrates that the fabricated microreactor channels and real-time monitoring could reduce the cost and increase the quality of the biodiesel [44].

3.5. Microfluidic device for quality assessment of blended biodiesel

The microfluidic devices are smaller and more sensitive compared to the similar range of biosensor [45]. Assessment of quality fuel is an essential step for commercialization. Petroleum products usage and changing climate has instigated to develop renewable fuels and use their blending mixtures (B-5, B-20 and E-20). Quality assessment of blending mixtures of biodiesel, ethanol and butanol can be measured using gravimetric measurements (density and viscosity [46]. The recent development of microfluidic device represents the integration of different electrical, mechanical and fluid manipulation; detection techniquesand analytical separation onto a single microchip for total analysis of the target [47]. Fabrication of microfluidic device with sensors based on MEMS technology has been developed. In this study, resonant microtubes embedded with in-line density meters and fuel cell sensors were fabricated and tested to determine gasoline, Fischer–Tropsch fuel, various blends of ethanol, butanol, diesel, biodiesel and contaminants like water and air. This prototype has demonstrated an in-built technology to monitor the quality of fuels, blending mixtures, biofuels, and other contaminants in a miniature form with reduced cost [46]. The microfluidic devices can also be used to determine air, water and food quality. The heavy metal, environment pollutant, toxins and contaminant can be real time monitored and this help in the optimization of biofuel and biodiesel process [48]. The optimization of biofuel production can be controlled by a single chip system made as Miniaturized bioreactors, this system's flow rate, temperature and oxygen can be monitored and controlled by these microfluidic devices. Han et al., developed a microalgae based device that was used for screening and monitoring of oil and biomass accumulation in various species [49].

The microfluidic devices can also be used to determine air, water and food quality. The heavy metal, environment pollutant, toxins and contaminant can be real time monitored and this help in the optimization of biofuel and biodiesel process [48]. The optimization of biofuel production can be controlled by a single chip system made as Miniaturized bioreactors, this system's flow rate, temperature and oxygen can be monitored and controlled by these microfluidic devices. Han et al., developed a microalgae based device that was used for screening and monitoring of oil and biomass accumulation in various species [49].

4. Microfludics in bioethanol technology

Bioethanol, in addition to biodiesel, is a prominent ‘drop-in’ fuel responsible for lower green house gas emissions [50]. Conventionally, bioethanol is mainly produced by yeast fermentation of sugars that are derived from sugar cane, maize, starchy crops or lignocellulosics [2]. However, technically several challenges have been confronted with

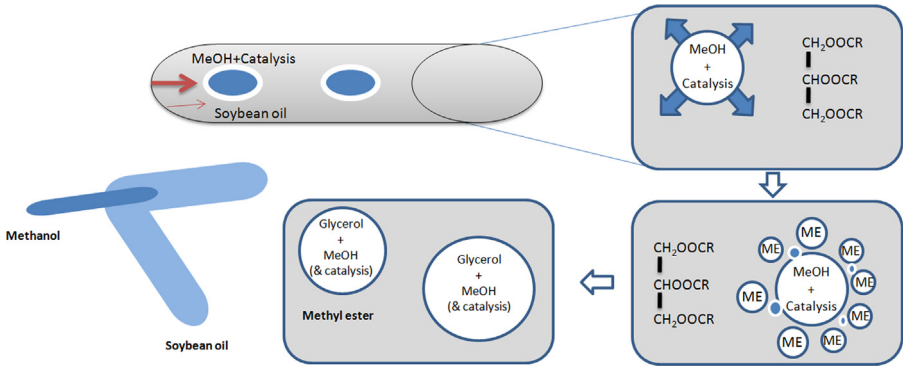

Fig. 2. Transesterification of soybean oil with methanol in microfluidic device (Adapted and modified from: Yeh et al. [20]).

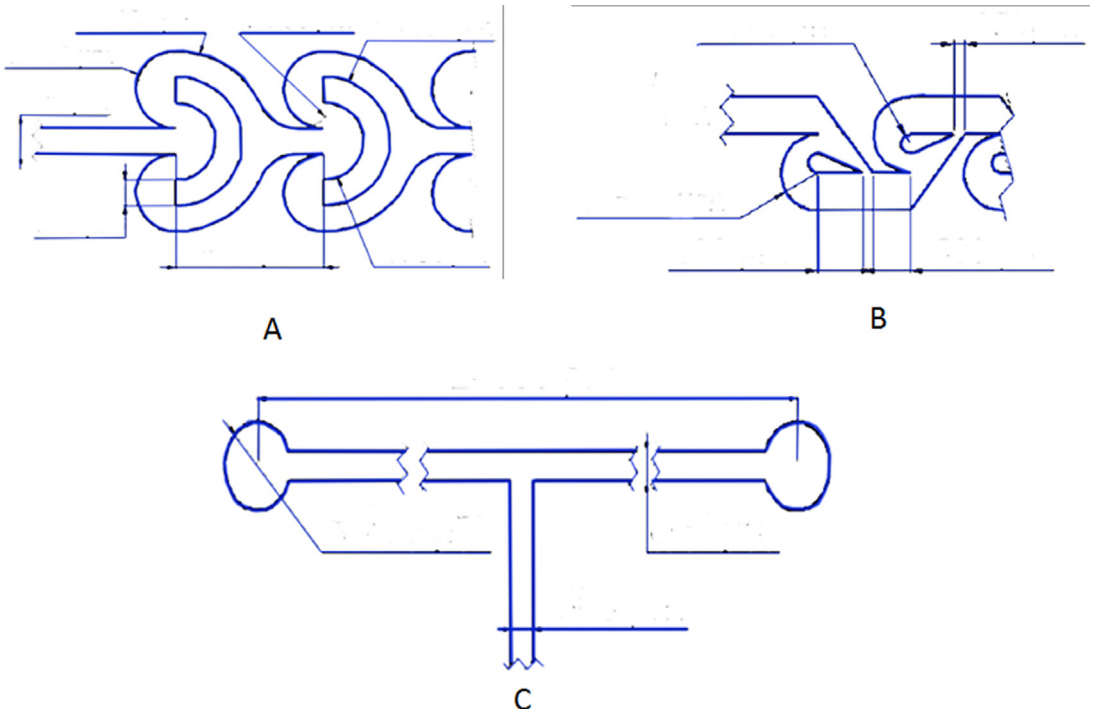

Fig. 3. Internal geometries of microreactors. A) Omega shape B) Tesla shape and C) T' shape (Adapted and modified from: Arias et al. [43]).

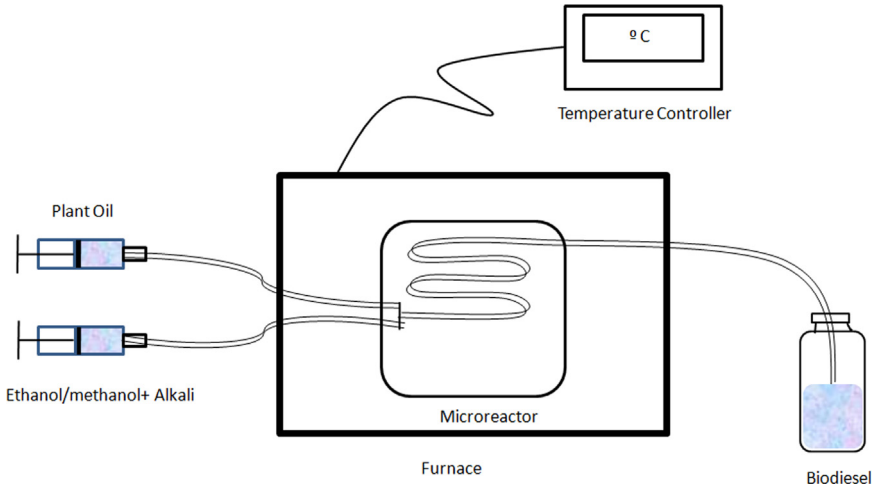

Fig. 4. Transesterification reaction set-up in microreactors (Adapted and modified from: Arias et al. [43]).

crop-based ethanol process such as food vs fuel controversy, poor utilization of pentoses, intolerance to osmotic stress and ethanol concentration [1]. To circumvent these problems, genetic and metabolic engineering have been attempted to develop a novel strain that utilize cellulose, xylose, simultaneous utilization of xylose and glucose, enhanced tolerance to ethanol concentration. Besides, genetic engineering have been envisaged in the 3rd generation feedstocks such as microalgae and cyanobacteria, which have potential as an alternative to crop-based ethanol feedstock by virtue of non-competitiveness with food chain, higher productivity and oxygenic photosynthesis [7]. However, after developing a transformant, screening of selective strain that have potential of higher ethanol productivity is essential. Abalde-Cela et al., [51] had developed a novel microfluidic droplet method to determine the efficiency in *Synechocystis* sp. strain PCC 6803. In this study, a microdroplet consists of enzymatic assay system that converts ethanol into fluorescent product ie., resorufin.

Microdroplets facilitate as a unique experiment and enables to identify genetically engineered cyanobacteria to discriminate ethanol producers from wild-type strains and also helpful in analysis of cyanobacteria library. In another study, utilizing droplet microfluidic technique the ethanol concentration during fermentation of *Zymomonas mobilis* has been determined with the enzyme assay of alcohol oxidase and horseradish peroxidase. Ethanol in presence of molecular oxygen can convert into acetaldehyde and hydrogen peroxide by alcohol oxidase. The hydrogen peroxide in-turn reacts with chromogen to form a dye and water by horseradish peroxidase enzyme. Unlike conventional determination of ethanol production using potassium dichromate method, this method showed higher sensitivity and reproducibility (a relative error of ethanol concentration < 5%), ethanol specific with meager consumption of chemicals and energy [52].

5. Development of microfludic reactors for biofuels production

5.1. Designing aspects of microfluIdic bioreactors

The recent discoveries in the field of micromachining and biotechnology allowed the optimization of biofuel production using controlled cell cultures. Microfluidics is a rapidly growing technology that allows control and manipulation of picoliter liquid using channel dimensions of little micrometers [53]. The use of microfluidic bioreactors for biofuel production is a promising upcoming method because of precise control. The microfluidic systems have bioreactors, which are comparable to the physical dimensions of cells and micro-organisms. For this reason, microfluidic bioreactors are ideal for studying the behavior of fuel producing cells and their characterization using micro-environment.

Microfluidic bioreactors have following advantages in comparison with the conventional biofuel production systems [54–56]:

- Controlled microenvironment
- Ease of integration and reduced cost
- Low initial seed of microorganisms
- Portability, automation, data logging, and analysis
- Use and throw chips
- Higher throughput and low time consumption
- Reduced sample volume requirements
- Ease of managing oxygen level, temperature, and PH

Effective microfluidic bioreactor design should consider three basic aspects of the manufacturing method, design considerations, and functional elements (Fig. 5).

5.2. Fabrication of microfludic bioreactors

The microfluidic bioreactor can be manufactured using following methods:

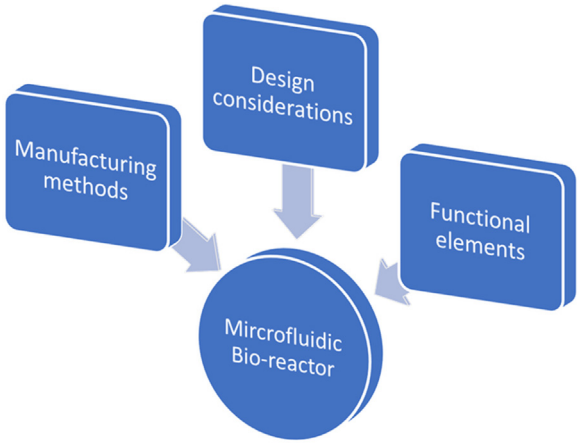

Fig. 5. Basic aspects of microfludic bioreactor design.

- Photolithography: In photolithography, the silicon wafer is etched to create channels and structures in the silicon wafer itself. This method is prevalent because of the traditional electronics device were made using the same photolithography method [57].
- Soft lithography: Soft-lithography involves master making using photo-lithography and then making actual devices by molding the elastomer like PDMS from the silicon master [58].
- Hot embossing: The bioreactor can be manufactured using thermoplastic and hot embossing. For Hot embossing, the negative of the pattern is manufactured in metal or hard substrate. Molten thermoplastic is pressed against the master and allowed to cool. Once the device is ready, it is ejected using ejector pin [59].
- Injection molding: This is a very popular technique in case of mass production of microfluidic bioreactors. Molten thermoplastic is injected against the master mold to make the microfluidic device [60].
- Direct milling: It is the traditional technique to manufacture devices by directly milling the device using high speed rotating tapered drill. The disadvantage with this direct milling method is the resolution of channels achieved is of the order of few hundreds of microns. Also, continuous wear of drill bits and heat generated due to friction are the other problems faced by this manufacturing method [61]. The typical steps involved in the fabrication of microfluidic bioreactor has shown in Fig. 6.

5.3. Parameters consideration for design of microfluidic bioreactor

Bio-compatibility of the material from which microfluidic bioreactor is made. The material should not be toxic to cells. Sterilization of microfluidic bioreactor is an important step. Microfluidic bioreactors are sterilized using solvents such as isopropanol Acetone, or Ethanol. After solvent evaporation, DI water rinse is given to the channels before media is loaded. With microfluidic bioreactors, inherent scaling effects such as the large surface area to volume ratio, low Reynolds number which results in laminar flows comes into the picture. Also, in microfluidic bioreactors, the viscous forces dominate the inertial forces [57,62].

5.4. Functional elements of microfluidic bioreactor

For automation of microfluidic bioreactor, functional elements are required. These functional elements are enlisted as follows:

a) Fluidic elements involve valves, pumps, mixers, and injectors (sequencers). Fluidic valves also called as quake vale controls [63] the flow of liquid inside the bioreactor. Fluidic pumps are used to control the flow rate inside the reactor. Syringe pumps are used. Peristaltic pump, piezo pump, and pressure pumps are popular

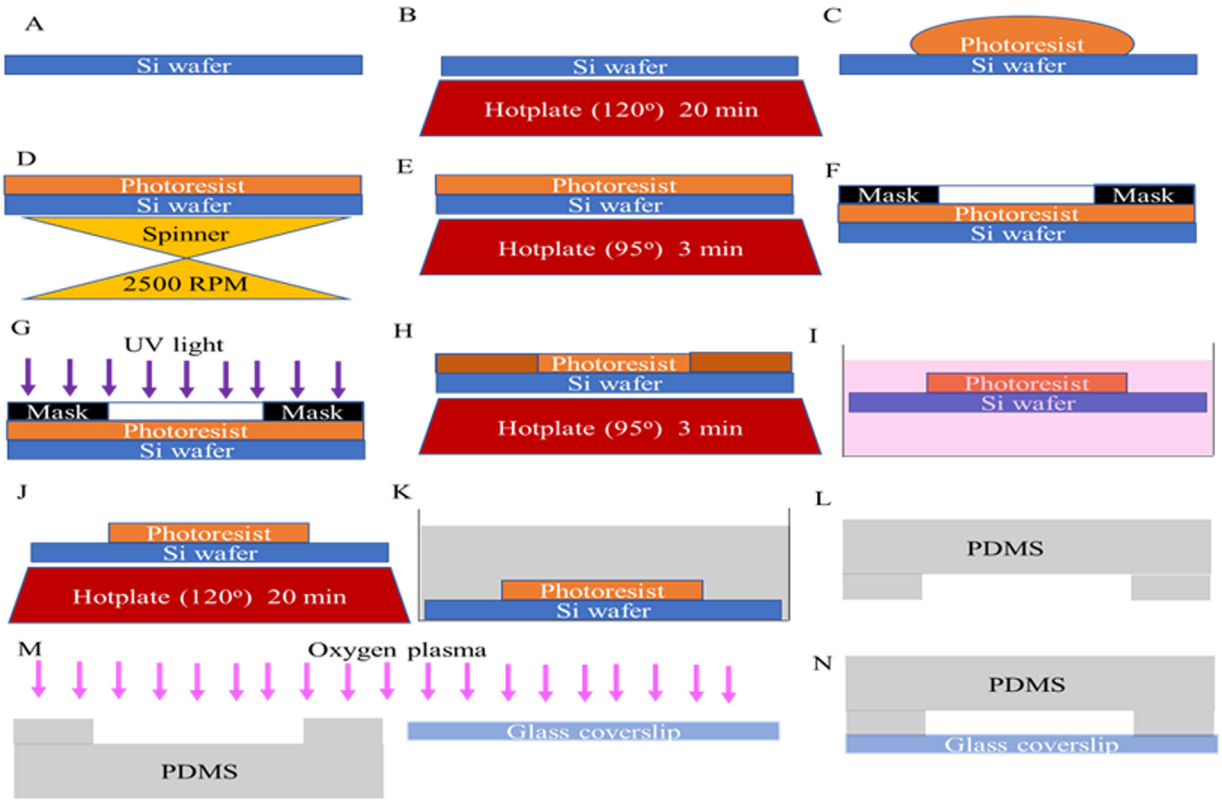

Fig. 6. Microfluidic bioreactor fabrication steps (A) Silicon (Si) wafer is RCA cleaned. (B) Si wafer is then heated at 120 °C for 20 min to remove any moisture. (C) SU-8 photoresist(PR) is poured on to the wafer. (D) The wafer is spun at 2500 RPM to ensure the uniform spread of PR. (E) The wafer is pre-baked at 95 °C for 3 min to remove any solvents. (F) Mask is placed on top of the wafer. (G) UV exposure is given to wafer. (H) Post-exposure bake is given at 95 °C for 3 min (I) The wafer is submerged into the SU-8 developer. (J) Hard bake is given at 120° for 20 min (K) Master is kept in a Petri dish, and freshly made PDMS is poured on to the master and degassed. (L) PDMS is cured, and the device is then cut taken out from the master. (M) Cleaned glass cover slip and PDMS device are exposed to oxygen plasma for 90 s (N) The device is then bonded to the glass cover slip.

nowadays [64]. With the microfluidicdevice, an inherent property of laminar flow exists. To overcome issues that might arrive with laminar flows are solved by mixers. Christmas tree design gradient generator [65] is the most popular mixer used with microfluidic bioreactors. Most of the automation is possible with microfluidic bioreactors are because of sequencers or injectors. The injector is programmable and defines which fluid should entire the device as per time.

b) Sensors are the feedback elements from bioreactors to achieve control action of the bioreactor. The most commonly used sensors are pH, O_2, and temperature [66,67]. The thermocoupleis popularly used as a temperature sensor because of small footprint and linear range. Other temperature sensors are a thermistor, RTD, etc. [68]. pH inside the device is measured using colorimetry test. In the colorimetric test buffer is flows which change color depending upon acidic or alkaline nature and magnitude. O_2 sensing is most important because it is a direct indicator of microorganism metabolism and hence the biofuel production rate. O_2 consumption can be related to metabolic reactions. O_2 inside a microfluidic bioreactor is measure using absorbance test or NMR spectroscopy.

c) Heating element: The most important functional element of the microfluidic bioreactor is the heating element. Resistive heating [69] is used, in which heat is controlled by controlling the current flowing through the resistive coil. Nowadays Peltier is used as a heating element because of its ability to heat and cool by just reversal of current. The problem with Peltier heating is it draws a large number of currents.

d) Gas exchange [70] is used in bioreactors to maintain certain concentration of gasses inside the microfluidic bioreactor chamber. Mostly oxygen and nitrogen are the two gasses used in gas exchange. The device made up with PDMS is useful in case of gas exchange because PDMS is permeable to air but non-permeable to liquids.

e) Simulations are the most basic functional element used in microfluidic bioreactors. As manufacturing masters using lithography is costly to process it is very important that the design should be optimized using computer simulations. COMSOL Multiphysics [71] and Ansys [72] are the two most popular simulation software used for simulation of the microfluidic bioreactor.

5.5. *Types of microfluidic bioreactors*

a) Membrane-based microfluidic bioreactor: In these bioreactors, a thin membrane is used to separate media and microorganism. Ferrell et al., [73] have developed a microfluidic membrane bioreactor for evaluation of renal epithelial cells.

b) Continuous stirrer microfluidic bioreactor: In these systems, the liquid is continuously stirred using piezo pumps. Gebhardt et al., [74] have designed milliliter-scale stirred tank bioreactors which used 48 wells and sample volume of 12 mL

c) Packed-bed microfluidic bioreactor: In packed bed microfluidic bioreactor catalyst particles are used to fill the microfluidic channel. Losey et al., [75] have used packed-bed bioreactor to analyze mass transfer and reaction. In a recent study, Trachsel and Philippe, [76] have used microfluidic packed-bed bioreactor for measurement of residence time distribution.

d) Microfluidic photobioreactor: Microfluidic photobioreactor uses phototropic microorganism and light source. These organism use photosynthesis to produce food. Kim et al., [24] used photobioreactor for high throughput screening of oil production.

Table 2
Comparison of different microfludic bioreactors.

	Advantages	Disadvantages	Impact	Ref
Membrane-based microfluidic bioreactor	Simple in construction Low power consumption	Issue of clogging Low time of operation	Output sample is filtered liquid Mamelian cells can be handled	[73]
Continuous stirrer microfluidic bioreactor	Good for mixing Large sample volume Simultaneous measurement	Continuous stirring can cause mechanical damage Inefficient in terms of power	Used for aerobic bioprocesses Suitable for Bioreactions with PCR	[74]
Packed-bed microfluidic bioreactor	Catalyst based reactions	Costly compared to other methods Beads involved causes particulate contamination	Used for fast bioreactions Resident time measurements	[75] [76]
Microfluidic photobioreactor	High throughput	Only specific to photonic reactions Light source and detector needed	Useful for photonic microorganisms Useful for plant cell based studies	[24]

The comparison of different microfluidic bioreactors have been summarized in Table 2.

6. Microfluidics in MFC and MEC technologies

In the past decade, remarkable progress is carried out in the field of microbial fuel cell technology for energy production, wastewater treatment and new product formation. This advance revolution in microbial fuel cell deals a multidisciplinary approach to achieve high efficiency and high wastewater treatment rates [6]. However, this technology is still at the lab scale, so for further understanding of this microfluidics plays an important role. The use of microfluidic in BES shows many advantages over macroscale system such as the need for less space and less time with the need for fewer reagents. MMFC which works based on the flow concepts in micro-environment is a new advance feature in MFC technology with the unique features of automation, robustness and specificity with the positive attributes of process accuracy and cost- effectiveness [77]. These unique and positive attributes are also coming with the less energy utilization with the standardized microfabricaton protocols with the ancillary benefits of microenvironment conditions such as high-surface area and flexible integration with other systems towards fully assembled platforms [78]. Owing to the unique multi-facet features and advantages, several researchers started to work on MMFC technologies as miniature setups as biosensor [79], environmental diagnostic tool [80,81], microbial screening miniature device [82] and as implantable pacemakers [83].

Zebda et al., investigated the microfabrication techniques tool to make a functional microfluidic glucose/O2 biofuel cell. In this study, oxygen is reduced by enzyme laccase at the cathode whereas glucose oxidase used to oxidize glucose at the anode. The Y-shaped design, giving an advantage of using different streams of oxidant and it prevents the anode from any interfering reaction with oxygen at anode electrode. The highest produced power in this system reached to 110 μW/cm^2 at 300 mV with 19 mM glucose at 23 °C. The maximum achieved current density was 690 μA/cm^2 at 300 mV. Compare to the standard miniaturization glucose fuel cell, this Y shaped microfluidic cell is more efficient [84].

In another research, Qian et al., demonstrated a unique 5-μL microfluidic MFC, this system generated a reproducible current generation. The bioelectricity generated by using *Shewanella oneidensis* MR-1 in the complex organic substrate. The maximum power produced in this system was 62.50 W/m^3. The maximum net power produced in this system was 250 mW and it was operated in fed-batch mode [85].

A membrane-less microfluidic MFC was devised by Wang et al., for detection of microbe's electroactivity. Maximum open circuit voltage obtained with different media such as activated microflora, untreated microflora and fresh medium were 246, 131 and 102 mV respectively. These obtained results show that microorganism added 115 mV compared to inactivated microflora (131 mV). This study proves that without proton exchange membrane μMFC, has the ability for detection of microbes [86]. A study on the effect of acetate concentration and flow rate in microfluidic MFC revealed a maximum power density of 618 mW/m^2 was achieved at the COD concentration of 1500 mg/L at the anolyte flow rate of 10 mL/h. The concerned results of higher power production using low internal resistance were attributed to the removal of proton exchange membrane and available high surface to volume ratio [87].

Selloum et al., first time demonstrated an ethanol microfluidic MFC based on bioanode and biocathode and operated in Y-shaped microfluidic channel. In the MMFC, at biocathode O_2 was reduced by laccase whereas at bioanode ethanol was oxidized by alcohol dehydrogenase. The maximum power density achieved was 90 μW/cm^2 at 600 mV for a flow rate of 16 μL/min. In this system, higher current and power densities due to the lower ohmic internal resistance [88]. A study on the effect of different microchannels (diverging, converging and vertical) on microfluidic MFC showed the superior results with diverging channel (a power density of 2.44 W/m^2) than the converging and straight channels. These results show that the geometry is critical for the higher performance of microfluidic MFC, due to converging geometry; lower anode resistance and good biofilm were formed in this microchannel geometry [89]. Escalona-Villalpando et al., investigated a glucose microfluidic MFC; this was constructed using glucose oxidase enzyme with supported on multiwall carbon nanotubes. The maximum power density of 610 μW/cm^2 was obtained with open circuit potential of 720 mV [90].

A novel laminar flow MFC array was demonstrated by Yang et al., as the scalable power source for portable lab-on-chip devices. The maximum generated power was 60.50 μW/cm^2 with 100 kΩ loads. This laminar based system did not require membrane between anode and cathode chambers; power was generated using *Pseudomonas aeruginosa* PAO1. Higher voltage was achieved by connecting these cells into the series mode [91]. Mardanpour et al., demonstrated the feasibility of microfluidic MFC for biohydrogen production and its usages for medical use. The use of nickel as electrode compared to the conventional electrode give better performance for power density and biohydrogen generation. The maximum produced power and biohydrogen were 2.2 μW/cm^2 and 1.4 μL H_2 μL substrate -1 day-1 respectively. The use of human excreta as the substrate in microfluidic MFC was tested first time with E Coli bacteria. The maximum hydrogen production with glucose was 0.84 μL hydrogen (μL glucose)-1 day-1, whereas 0.94 μL hydrogen (μL urea)-1 day-1 from urea. Theoretical investigation shows that more substrate reduction was occurred from a biofilm on electrode surface compared to suspended bacteria [92].

Pramanik et al., investigated a microfluidic MFC for $NaBH_4$ electrooxidation with a different operating condition such as temperature, pressure, electrolyte concentration, electrode surface area, Pt loading and gas diffusion layer. The maximum power density, current density and open circuit voltage were 24.09 mW/m^2, 54.97 mA/m^2 and 1079 mV respectively. These were obtained at the temperature of 70 °C, with Pt loading on the electrode of 1 mg/cm^2 with 0.1 M $NaBH_4$ mixed with 1 M KOH as an electrolyte [93]. In another study by Jiang et al., utilized a miniaturized microfluidic MFC with porous graphene foam and separated by a proton exchange membrane with a current collector at the bottom of anode chamber. The maximum obtained volumetric

Table 3
Overview of fabricated Microfluidics in MFC devices and their main characteristics.

Material	Microbes	Anode volume	Anode area	Catholyte	Cathode specific area	PEM area	Electrode distance	Pmax (W/m^3)	I_{max} (A/m^3)	Imax (mA/m^2)	Reference
Plastic	Geobacter sulfurreducens	7 mL	Gold (7.8 cm^2)	N/A	Graphite cloth (7.8 cm^2)	7.8 cm^2	1.7 cm	N/A	76.66	688	[95]
Plastic	Mixed bacterial culture	2.5 mL	Carbon cloth (7 cm^2)	Air	Carbon cloth (7 cm^2)	7 cm^2	1.7 cm	1010	5050	9000	[96]
Plastic	Shewanella oneidensis DSP-10	1.2 mL	RVC (37 cm^2)	FeCN	RVC (37 cm^2)	2 cm^2	175 lm	660	1800	42.162	[97]
Poly(dimethylsiloxane	–	–	gold 0.05 cm2	laccase (0.5 mg mL^{-1}) and ABTS (5 mM)	NA	NA	NA	110 $lWcm^{-2}$	NA	690 μA cm_2	[84]
Poly(dimethylsiloxane	*S. oneidensis MR-1*	4 μL	0.4 cm^2 (carbon cloth)	FeCN	NA	NA	NA	62.5Wm^{-3}	NA	10 $\mu A/cm^2$	[85]
Poly(dimethylsiloxane	*S. oneidensis MR-1*	10 mL	5 cm^2 (carbon cloth)	FeCN	NA	NA	NA	0.25Wm^{-3}	NA	8 $\mu A/cm^2$	[85]
Poly(dimethylsiloxane	*S. oneidensis MR-1*	10 mL	2.25 cm2 (gold)	Air	NA	NA	NA	NA	NA	NA	[85]
Poly(dimethylsiloxane	*S. oneidensis MR-1*	1.5 μL	0.15 cm^2 (gold)	FeCN	NA	NA	NA	15 Wm^{-3}		13 $\mu A/cm^2$	[85]
PDMS	S. oneidensis MR-1 and 7Ca	435 μL	0.385 cm^2	FeCN	0.385 cm2	NA	NA	NA	NA	150	[98]
PDMS	G. sulfurreducens	4.5 μL	2.25 cm^2	FeCN	NA	2.25	480 μm	2300 μW/cm3	NA	330	[99]
Glass/PDMS/PTEEe	G. sulfurreducens	15.5 μL	1 cm^2	FeCN	1 cm2	1	NA	NA	NA	260	[100]

power density and surface power density of 745 $\mu W/cm^3$ and 89.40 $\mu W/cm^2$ respectively. The *Shewanella oneidensis* was used as a model organism in this study [94]. The series or parallel connection of microfluidic MFC, integrated with the lab on chip device offers a significant application in real life applications this. With these connections, higher power and cell voltage can be achieved and these can be easily scalable. The noteworthy research findings of microfluidics-based MFC technologies have been tabulated under Table 3. The microfluidic MFCs technology in current structure needs more improvement for translating it to practical integration.

7. Future prospects

The positive attributes of microfluidics have to be envisaged to integrate the whole laboratory (lab-on-a-chip), automating genetic engineering and perform all functions in a single chip/device. The other research domain which has to be envisaged is the development of novel miniature sophisticated analytical interface in a high-throughput fashion to monitor the biofuel raw material quality and online/in-vivo monitoring of the biofuel production conditions. Moreover, the contamination aspects of microalgae under microfluidic conditions and its suitability for co-culturing of bacteria with microalgae have to be envisaged for a successful biofuel platform which was not successful with the previous research attempts [25]. Significant research has to be explored to immobilize the microbes on microfluidic devices and usage of mediator molecules towards the attainment of higher energy efficiencies either individually or in parallel [38]. Furthermore, pertaining to MMFC technologies, more research studies have to be conducted to optimal microfluidic environmental conditions such as a dynamic pattern of microfluidics, flow and transport of microfluidics in sandwiched configurations and microfluid behavior in different zonal and boundary conditions [77]. More efforts have to done to develop a multiplexed pilot scale MMFC technology for energy harvesting by focusing on the flow-kinetic modelling and simulation to achieve a continuous co-laminar flow and by attaining the minimum internal resistance in MMFC's [77]. Notwithstanding, the viability of these technologies should be evaluated at pilot-scale operations and to encourage these technology driven-process in bioenergy and biofuel process, significant impetus should be devoted while framing policies in the form of subsidies, incentives for effective adoption.

8. Conclusion

Recent evidence on microfluidics applications in bioenergy and biofuel domains showcase the potential to develop cost-effective and automated process. These technologies have shown tremendous opportunity in reducing time, energy, solvent consumption with precision results for efficient and inexpensive biodiesel/bioethanol production. The miniature-MMFC's seems to be a viable solution as power machines, biosensors, implantable medical devices and as environmental monitoring systems. More research is anticipated to develop an integrated chip/automated process performing all operations pertaining to a process to ascertain the quality of the fuel/product. Further, the inclusion of these technologies should be encouraged while formulating energy policies. Overall, the unique features of microfluidics-based technologies could envisage the need for cutting-edge technologies towards up gradation of biofuel and bioenergy sectors.

Acknowledgement

RB would like to thank IIT Kharagpur, India for providing the resource amenities for writing the review draft. JKSP is grateful to IIT Kharagpur, India and ICAR-Indian Institute of Seed Science, Mau, India for providing resource facilities to execute the present review article. SS and NM are thankful to IIT Guwahati, India and IIT Mumbai, India, respectively for utilization of learning resources. VKG is very grateful to

the Jaypee University of Information Technology, India for providing the research amenities and learning resources to prepare and successful execution of the review draft.

Conflict of interest

The authors declared that they have no conflict of interests in publishing this article.

References

[1] Chandel AK, Kumar VK, Singh AK, Antunes FAF, da Silva SS. The path forward for lignocellulose biorefineries: bottlenecks, solutions, and perspective on commercialization. Bioresour Technol 2018;264:370–81.
[2] Nizami AS, Rehan M, Waqas M, Naqvi M, Ouda OKM, Shahzad K, Miandad R, Khan MZ, Syamsiro M, Ismail IMI, Pant D. Waste biorefineries: enabling circular economies in developing countries. Bioresour Technol 2017;241:1101–17.
[3] Kumar SPJ, Banerjee R. Enhanced lipid extraction from oleaginous yeast biomass using ultrasound assisted extraction: a greener and scalable process. UltrasonSonochem 2018. https://doi.org/10.1016/j.ultsonch.2018.08.003.
[4] Kumari A, Mahapatra P, Garlapati VK, Banerjee R. Enzymatic transesterification of Jatropha oil. Biotechnol Biofuels 2009;2(1):1.
[5] Kumar SPJ, Garlapati VK, Dash A, Scholz P, Banerjee R. Sustainable green solvents and techniques for lipid extraction from microalgae: a review. Algal Res 2017;21:138–47.
[6] Pant D, Bogaert GV, Diels L, Vanbroekhoven K. A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresour Technol 2010;101(6):1533–43.
[7] Singh A, Pant D, Korres NE, Nizami AS, Prasad S, Murphy JD. Key issues in life cycle assessment of ethanol production from lignocellulosic biomass: challenges and perspectives. Bioresour Technol 2010;101(13):5003–12.
[8] Ambat I, Srivastava V, Sillanpää M. Recent advancement in biodiesel production methodologies using various feedstock: a review. Renew Sustain Energy Rev 2018;90:356–69.
[9] Garlapati VK, Kant R, Kumari A, Mahapatra P, Das P, Banerjee R. Lipase mediated transesterification of Simarouba glauca oil: a new feedstock for biodiesel production. Sustain Chem Process 2013;1:11.
[10] Beebe DJ, Mensing GA, Walker GM. Physics and applications of microfluidics in biology. Annu Rev Biomed Eng 2002;4:261–86.
[11] Zhang J, Yan S, Yuan D, Alici G, Nguyen NT, Ebrahimi Warkiani M, Li W. Fundamentals and applications of inertial microfluidics: a review. Lab Chip 2016;16(1):10–34.
[12] Brody J, Yager P. Diffusion-based extraction in a microfabricated device. Sens Actuators A 1997;A58:13–8.
[13] Hatch A, Kamholz AE, Hawkins KR, Munson MS, Schilling EA, Weiglm BH, Yager P. A rapid diffusion immunoassay in a T-sensor. Nat Biotechnol 2001;2001(19):461–5.
[14] Mahesh K, Vaidya S. Microfluidics: a boon for biological research. Curr Sci 2017;112(10):2021–8.
[15] Jacobson S, McKnight T, Ramsey J. Microfluidic devices for electrokinetically driven parallel and serial mixing. Anal Chem 1999;71:4455–9.
[16] Liu R, Stremler M, Sharp K, Olsen M, Santiago J, et al. Passive mixing in a three-dimensional serpentine microchannel. J Microelectromec Syst 2000;9:190–7.
[17] Dertinger S, Chiu D, Jeon N, Whitesides G. Generation of gradients having complex shapes using microfluidic networks. Anal Chem 2001;73:1240–6.
[18] Jeon N, Dertinger S, Chiu D, Choi I, Stroock A, Whitesides G. Generation of solution and surface gradients using microfluidic systems. Langmuir 2000;16:8311–6.
[19] Vowell S. Microfluidics: effects of surface tension. Physics 2009;486:1–10.
[20] Yeh SI, Huang YC, Cheng CH, Cheng CM, Yang JT. Development of a millimetrically scaled biodiesel transesterification device that relies on droplet-based co-axial fluidics. Sci Rep 2016;6:29288.
[21] Kumar SPJ, Banerjee R. Optimization of lipid enriched biomass production from oleaginous fungus using response surface methodology. Ind J Exp Biol 2013;51(11):979–83.
[22] Sackmann EK, Fulton AL, Beebe DJ. The present and future role of microfluidics in biomedical research. Nature 2014;507(7491):181–9.
[23] Shih SC, Goyal G, Kim PW, Koutsoubelis N, Keasling JD, Adams PD, Hillson NJ, Singh AK. A versatile microfluidic device for automating synthetic biology. ACS Synth Biol 2015;4(10):1151–64.
[24] Kim HS, Weiss TL, Thapa HR, Devarenne TP, Han A. A microfluidic photobioreactor array demonstrating high-throughput screening for microalgal oil production. Lab Chip 2014;14(8):1415–25.
[25] Kim HS, Devarennec TP, Han A. Microfluidic systems for microalgal biotechnology: a review. Bioresour Technol 2018;30:149–61.
[26] Bartley ML, Boeing WJ, Corcoran AA, Holguin FO, Schaub T. Effects of salinity on growth and lipid accumulation of biofuel microalga *Nannochloropsis salina* and invading organisms. Biomass-Bioenergy 2013;54:83–8.
[27] Syed SM, Rafeie M, Vandamme D, Asadnia M, Henderson R, Taylor RA, Warkiani ME. Selective separation of microalgae cells using inertial microfluidics. Bioresour Technol 2017;252:91–9.
[28] Barros AI, Gonçalves AL, Simões M, Pires JCM. Harvesting techniques applied to microalgae: a review. Renew Sustain Energy Rev 2015;41:1489–500.
[29] Ahmad AL, Yasin NHM, Derek CJC, Lim JK. Comparison of harvesting methods for microalgae Chlorella sp. and its potential use as a biodiesel feedstock. Environ Technol 2014;35(17):2244–53.
[30] Al-Hothaly KA, Adetutu EM, Taha M, Fabbri D, Lorenzetti C, Conti R, May BH, Shar SS, Bayoumi RA, Ball AS. Bio-harvesting and pyrolysis of the microalgae *Botryococcus braunii*. Bioresour Technol 2015;191:117–23.
[31] Hønsvall BK, Altin D, Robertson LJ. Continuous harvesting of microalgae by new microfluidic technology for particle separation. Bioresour Technol 2015;200:360–5.
[32] Maa F, Hanna AM. Biodiesel production: a review. Bioresour Technol 1999;70:1–15.
[33] Neethirajan S, Kobayashi I, Nakajima M, Wu D, Nandagopal S, Lin F. Microfluidics for food, agriculture and biosystems industries. Lab Chip 2011;11:1574–86.
[34] Whitesides GM. The origins and the future of microfluidics. Nature 2006;442:368–73.
[35] Oncescu V, Erickson D. High volumetric power density, non-enzymatic, glucose fuel cells. Sci Rep 2013;3:1226.
[36] Yeh SI, Huang YC, Cheng CH, Cheng CM, Yang JT. Development of a millimetrically scaled biodiesel transesterification device that relies on droplet-based co-axial fluidics. Sci Rep 2016;6:29288.
[37] Uhlen M, Svahn HA. Lab on a chip technologies for bioenergy and biosustainability research. Lab Chip 2011;11:3389.
[38] Lee JW, Kjeang E. A perspective on microfluidic biofuel cells. Biomicrofluidics 2010;4(4):041301.
[39] European Standard CEN EN 14214. Requirements and test methods; 2008.
[40] ASTM Standard D6751-11a. American standards for testing and materials (ASTM), West Conshohocken, PA; 2011.
[41] Qiu Z, Zhao L, Weatherley L. Process intensification technologies in continuous biodiesel production. Chem Eng Process 2010;49:323.
[42] Stiefel S, Dassori G. Simulation of biodiesel production through transesterification of vegetable oils. Ind Eng Chem Res 2009;48:1068–71.
[43] Arias ELM, Martins PF, Munhoz AJ, Gutierrez-Rivera L, Filho RM. Continuous synthesis and in situ monitoring of biodiesel production in different microfluidic devices. Ind Eng Chem Res 2012;51:10755–67.
[44] Chuck CJ, Bannister CD, Hawley JG, Davidson MG. Spectroscopic sensor techniques applicable to real-time biodiesel determination. Fuel 2010;89:457–61.
[45] Ligler FS, Anderson GP, Davidson PT, Foch RJ, Ives JT, King KD, Page G, Stenger DA, Whelan JP. Remote sensing using an airborne biosensor. Environ Sci Technol 1998;32(16):2461–6.
[46] Sparks D, Smith R, Riley D, Tran N, Patel J, Chimbayo A, Najafi N. Monitoring and blending biofuels using a microfluidic sensor. J ASTM Int 2010;7(8):1–9.
[47] Fiorini GS, Chiu DT. Disposable microfluidicdevices: fabrication, function, and application. Biotechniques 2005;38:429–46.
[48] Saxena K, Jain S, Sharma DK, Dua R, Kamthania M. Applications of integrated microfluidic devices in environmental monitoring: a review. J Energy Environ Sci Photon 2014;128:521–30.
[49] Han A, Hou H, Li L, Kim HS, de Figueiredo P. Microfabricated devices in microbial bioenergysciences. Trends Biotechnol 2013;31(4):225–32.
[50] Chintagunta AD, Ray S, Banerjee R. An integrated bioprocess for bioethanol and biomanure production from pineapple leaf waste. J Clean Prod 2017;165. [1508-151].
[51] Abalde-Cela S, Gould A, Liu X, Kazamia E, Smith AG, Abell C. High-throughput detection of ethanol producing cyanobacteria in a microdroplet platform. J R Soc Interface 2015;12:20150216.
[52] Churski K, Ruszczak A, Jakiela S, Garstecki P. Droplet microfluidic technique for the study of fermentation. Micromachines 2015;6:1514–25.
[53] Ma X, Huo YX. The application of microfludic-based technologies in the cycle of metabolic engineering. Synth Syst Biotechnol 2016;1(3):137–42.
[54] Stadler P, Farnleitner AH, Zessner M. Development and evaluation of a self-cleaning custom-built auto sampler controlled by a low-cost Raspberry *Pi* microcomputer for online enzymatic activity measurements. Talanta 2017;162:390–7.
[55] Nourmohammadzadeh M, Lo JF, Bochenek M, Mendoza-Elias JE, Wang Q, Li Z, Zeng L, Qi M, Eddington DT, Oberholzer J, Wang Y. Microfluidic array with integrated oxygenation control for real-time live-cell imaging: effect of hypoxia on physiology of microencapsulated pancreatic islets. Anal Chem 2013;85(23):11240–9.
[56] Yang W, Woolley AT. Integrated multiprocess microfluidic systems for automating analysis. JALA Charlottesv Va 2010;15(3):198–209.
[57] Pasirayi G, Auger V, Scott SM, Rahman PKSM, Islam M, O`Hare L, Ali Z. Microfluidic bioreactors for cell culturing: a review. Micro Nanosyst 2011;3(2):137–60.
[58] Christoffersson J, Bergström G, Schwanke K, Kempf H, Zweigerdt R, Mandenius CF. A microfluidic bioreactor for toxicity testing of stem cell derived 3D cardiac bodies. Methods Mol Biol 2016;1502:159–68.
[59] Malahat S, Iovenitti PG, Sbarski I. Influence of tool fabrication process on characteristics of hot embossed polymer microfluidic chips for electrospray. J Microsyst Technol 2010;16(12):2075–85.
[60] Mair DA, Geiger E, Pisano AP, Fréchet JM, Svec F. Injection molded microfluidic chips featuring integrated interconnects. Lab Chip 2006;6(10):1346–54.
[61] Hsieh YK, Chen SC, Huang WL, Hsu KP, Gorday KAV, Wang T, Wang J. Direct micromachining of microfluidic channels on biodegradable materials using laser ablation. Polymers 2017;9:242.
[62] Das T, Chakraborthy S. Biomicrofluidics: recent trends and future challenges. Sadhana 2009;34(4):573–90.
[63] Thorsen T, Maerkl SJ, Quake SR. Microfluidic large-scale integration. Science 2002;298(5593):580–4.
[64] Skafte-Pedersen P, Sabourin D, Dufva M, Snakenborg D. Multi-channel peristaltic pump for microfluidic applications featuring monolithic PDMS inlay. Lab Chip

2009;9(20):3003–6.
[65] Somaweera H, Ibraguimov A, Pappas D. A review of chemical gradient systems for cell analysis. Anal Chim Acta 2016;907:7–17.
[66] Lee KS, Boccazzi P, Sinskey AJ, Ram RJ. Microfluidic chemostat and turbidostat with flow rate, oxygen, and temperature control for dynamic continuous culture. Lab Chip 2011;11(10):1730–9.
[67] Lee HL, Boccazzi P, Ram RJ, Sinskey AJ. Microbioreactor arrays with integrated mixers and fluid injectors for high-throughput experimentation with pH and dissolved oxygen control. Lab Chip 2006;6(9):1229–35.
[68] Dames C. Resistance temperature detectors. In: Li D, editor. Encyclopedia of microfludics and nanofludics. Boston: Springer; 2008. p. 68–86.
[69] McCreedy T, Wilson NG. Microfabricated reactors for on-chip heterogeneous catalysis. Analyst 2001;126(1):21–3.
[70] Cimetta E, Figallo E, Cannizzaro C, Elvassore N, Vunjak-Novakovic G. Micro-bioreactor arrays for controlling cellular environments: design principles for human embryonic stem cell applications. Methods 2009;47(2):81–9.
[71] Jiang H, Weng X, Li D. A novel microfluidic flow focusing method. Biomicrofluidics 2014;8(5):054120.
[72] Chen PC, Fan W, Hoo TK, Chan LCZ, Wang Z. Simulation guided-design of a microfludic thermal reactor for polymerase chain reaction. Chem Eng Res Des 2012;90(5):591–9.
[73] Ferrell N, Desai RR, Fleischman AJ, Roy S, Humes HD, Fissell WH. A microfluidic bioreactor with integrated transepithelial electrical resistance (TEER) measurement electrodes for evaluation of renal epithelial cells. Biotechnol Bioeng 2010;107(4):707–16.
[74] Gebhardt G, Hortsch R, Kaufmann K, Arnold M, Weuster-Botz D. A new microfluidic concept for parallel operated milliliter-scale stirred tank bioreactors. Biotechnol Prog 2011;27(3):684–90.
[75] Losey MW, Schmidt MA, Jensen KF. Microfabricated multiphase packed-bed reactors: characterization of mass transfer and reactions. Ind Eng Chem Res 2001;40(12):2555–62.
[76] Trachsel F, Philippe RVR. Residence time distribution in a packed bed microreactor. In: Proceedings of the 2006 AIChE annual meeting. American Institute of Chemical Engineers (AIChE); 2006.
[77] Goel S. From waste to watts in micro-devices: review on development of membraned and membrane less microfluidic microbial fuel cell. Appl Mater Today 2018;11:270–9.
[78] Kirby J. Micro- and Nanoscale Fluid Mechanics. Cambridge University Press; 2013.
[79] Li F, Zheng Z, Yang B, Zhang X, Li Z, Lei L. A laminar-flow based microfluidic microbial three-electrode cell for biosensing. Electrochim Acta 2016;199:45–50.
[80] Lee H, Yang W, Wei X, Fraiwan A, Choi S. A microsized microbial fuel cellbased biosensor for fast and sensitive detection of toxic substances in water. In: Proceedings of the 28th IEEE international conference on paper presented at the microelectro mechanical systems (MEMS); 2015.
[81] Qian F, He Z, Thelen MP, Li Y. A microfluidic microbial fuel cell fabricated bysoft lithography. Bioresour Technol 2011;102(10):5836–40.
[82] Mukherjee S, Su S, Panmanee W, Irvin RT, Hassett DJ, Choi S. A microliter-scale microbial fuel cell array for bacterial electrogenic screening. Sens Actuators A Phys 2013;201:532–7.
[83] Ren H, Lee HS, Chae J. Miniaturizing microbial fuel cells for potential portable power sources: promises and challenges. Microfluid Nanofluid 2012;13(3):353–81.
[84] Zebda A, Renaud L, Cretin M, Pichot F, Innocent C, Ferrigno R, Tingry S. A microfluidic glucose biofuel cell to generate micropower from enzymes at ambient temperature. Electrochem Commun 2009;11(3):592–5.
[85] Qian F, He Z, Thelen MP, Li Y. A microfluidic microbial fuel cell fabricated by soft lithography. Biorem J 2011;102:5836–40.
[86] Wang HW, Su JY. Membraneless microfluidic microbial fuel cell for rapid detection of electrochemical activity of microorganism. Bioresour Technol 2013;145:271–4.
[87] Ye D, Yang Y, Li J, Zhu X, Liao Q, Denb B, Chen R. Performance of a microfluidic microbial fuel cell based on graphite electrodes. Int J Hydrog Energy 2013;38:15710–5.
[88] Selloum D, Tingry S, Techer V, Renaud L, Innocent C, Zouaoui A. Optimized electrode arrangement and activation of bioelectrodes activity by carbon nanoparticles for efficient ethanol microfluidic biofuel cells. J Power Source 2014;269:834–40.
[89] Yang Y, Ye D, Li J, Zhu X, Liao Q, Zhang B. Biofilm distribution and performance of microfluidic microbial fuel cells with different microchannel geometries. Int J Hydrog Energy 2015;40(35):11983–8.
[90] Escalona-Villalpando RA, Dector A, Dector D, Moreno-Zuria A, Duron-Torres SM, Galvan-Valencia M, Arriaga LG, Ledesma-Garcıa J. Glucose microfluidic fuel cell using air as oxidant. Int J Hydrog Energy 2016;41:23394–400.
[91] Yang W, Lee KK, Choia S. A laminar-flow based microbial fuel cell array. Sens Actuators B Chem 2017;243:292–7.
[92] Mardanpour MM, Yaghmaei S. Dynamical analysis of microfluidic microbial electrolysis cell via integrated experimental investigation and mathematical modeling. Electrochim Acta 2017;227:317–29.
[93] Pramanik H, Rathoure AK. Electrooxidation study of $NaBH_4$ in a membraneless microfluidic fuel cell with air breathing cathode for portable power application. Int J Hydrog Energy 2017;42:5340–50.
[94] Jiang H, Ali MA, Xu Z, Halverson LJ, Dong L. Integrated microfluidic flow-through microbial fuel cells. Sci Rep 2017;7:41208.
[95] Richter H, Mccarthy K, Nevin KP, Johnson JP, Rotello VM, Lovley DR. Electricity generation by *Geobacter sulfurreducens* attached to gold electrodes. Langmuir 2008;24:4376–9.
[96] Fan Y, Hu H, Liu H. Enhanced Coulombic efficiency and power density of air-cathode microbial fuel cells with an improved cell configuration. J Power Sources 2007;171(2):348–54.
[97] Ringeisen BR, Henderson E, Wu PK, Pietron J, Ray R, Little B, et al. High power density from a miniature microbial fuel cell using *Shewanella oneidensis* DSP10. Environ Sci Technol 2006;40(8):2629–34.
[98] Hou H, Li L, Cho Y, de Figueiredo P, Han A. Microfabricated microbial fuel cell arrays reveal electrochemically active microbes. PloS One 2009;4(8):e6570.
[99] Choi S, Lee H, Yang Y, Parameswaran P, Rittmann BE, Chae J. A μL-scale micromachined microbial fuel cell having high power density. Lab Chip 2011;11(6):1110–7.
[100] Choi S, Chae J. Optimal biofilm formation and power generation in a micro-sized microbial fuel cell (MFC). Sens Actuators A Phys 2013;195:206–12.