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Review

A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production

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ABSTRACT

Microbial fuel cells (MFCs) have gained a lot of attention in recent years as a mode of converting organic waste including low-strength wastewaters and lignocellulosic biomass into electricity. Microbial production of electricity may become an important form of bioenergy in future because MFCs offer the possibility of extracting electric current from a wide range of soluble or dissolved complex organic wastes and renewable biomass. A large number of substrates have been explored as feed. The major substrates that have been tried include various kinds of artificial and real wastewaters and lignocellulosic biomass. Though the current and power yields are relatively low at present, it is expected that with improvements in technology and knowledge about these unique systems, the amount of electric current (and electric power) which can be extracted from these systems will increase tremendously providing a sustainable way of directly converting lignocellulosic biomass or wastewaters to useful energy. This article reviews the various substrates that have been explored in MFCs so far, their resulting performance, limitations as well as future potential substrates.

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1. Microbial fuel cells (MFCs) – an introduction

Microbial fuel cells (MFCs) have emerged in recent years as a promising yet challenging technology. In a MFC, microorganisms interact with electrodes using electrons, which are either removed or supplied through an electrical circuit (Rabaey et al., 2007). MFCs are the major type of bioelectrochemical systems (BESs) which convert biomass spontaneously into electricity through the metabolic activity of the microorganisms. MFC is considered to be a promising sustainable technology to meet increasing energy needs, especially using wastewaters as substrates, which can generate electricity and accomplish wastewater treatment simultaneously, thus may offset the operational costs of wastewater treatment plant (Lu et al., 2009). The knowledge that bacteria can generate electric current was first reported by Potter (1911). However, the real interest in MFCs has tremendously grown in recent years, both in terms of number of researchers as well as the applications for these systems. Fig. 1A shows that 'Scopus' search with keyword "microbial fuel cell" indicates almost 60-fold increase in the number of articles published over the last one decade (1998–2008). Moreover, the reported electric current output from the MFCs has also increased tremendously over the recent years. Fig. 1B shows the country-wise distribution of MFC researchers, the data for which was also drawn from 'Scopus'. It is evident that the inter-

est in MFC research is truly global with more and more researchers coming up from different countries.

Over the past years, MFCs as a new source of bioenergy have been extensively reviewed. These include information on the various terminology and measurements used in these systems (Logan et al., 2006), state of the art information on MFCs and recent improvements in MFC technologies (Du et al., 2007), comparison of MFCs with conventional anaerobic digestion (Pham et al., 2006), practical implementation of BESs (Rozendal et al., 2008), bioanode performance in BES (Pham et al., 2009b), cathodic limitations in MFCs (Rismani-Yazdi et al., 2008). The mechanism of external electron transfer from two main bacteria in BES studies, *Geobacter sulfurreducens* and *Shewanella oneidensis* was described in great detail by Debabov (2008). 'Microbial fuel cells' (Logan, 2008) is another source of comprehensive information on MFCs. Logan (2009) presented the power densities for MFCs, normalized to electrode-projected surface areas reported over the years 1998–2008.

However, a comprehensive review on the various substrates which have been used and can possibly be used in MFCs is still lacking. Substrate is important for any biological process as it serves as carbon (nutrient) and energy source. The efficiency and economic viability of converting organic wastes to bioenergy depend on the characteristics and components of the waste material. Especially the chemical composition and the concentrations of the components that can be converted into products or fuels, is of major interest while considering the potential substrates in BES systems (Angenent and Wrenn, 2008). The substrate influences not

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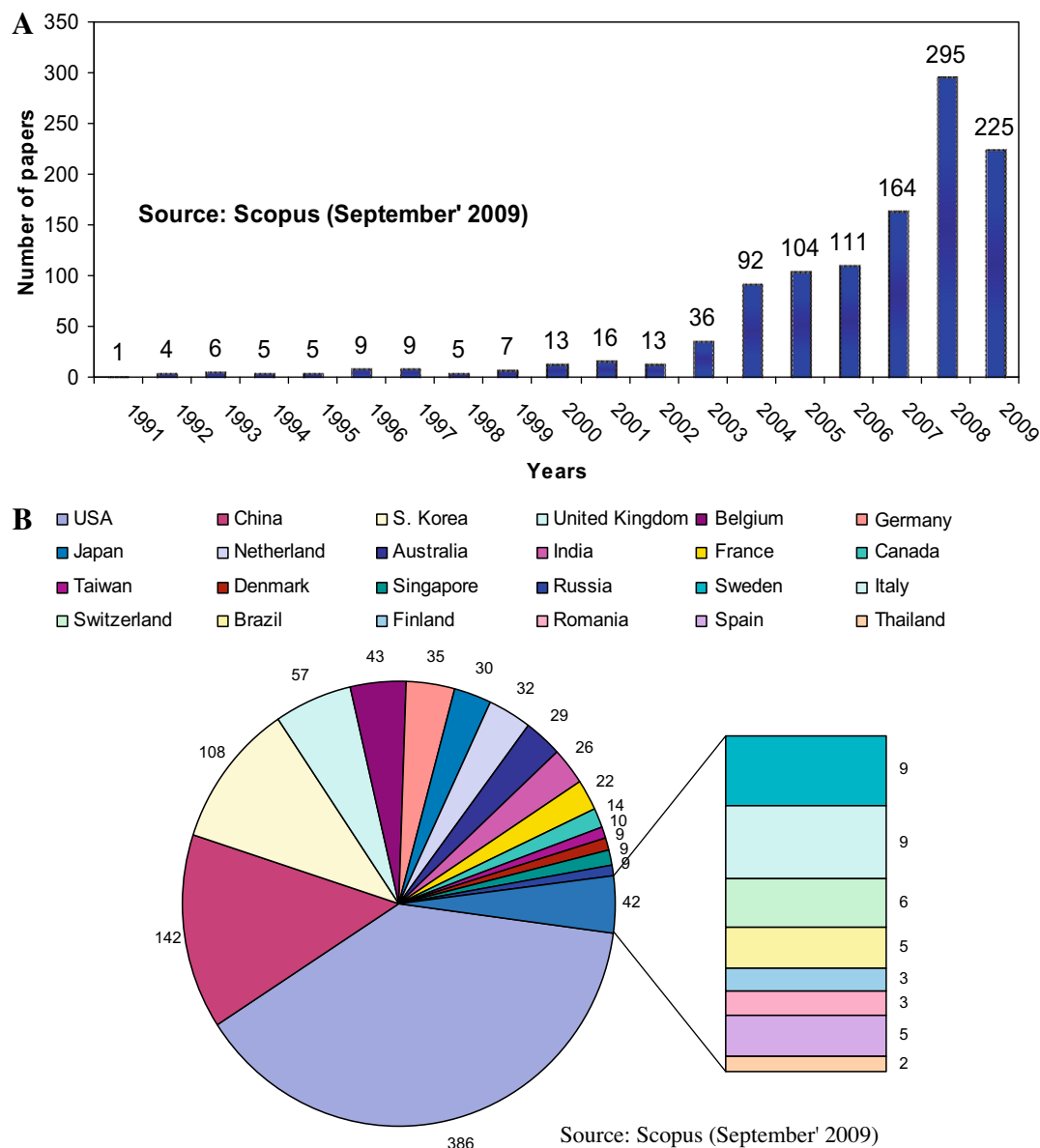


Fig. 1. (A) The number of articles on MFCs. The data is based on the number of articles mentioning MFC in the citation database Scopus in September' 2009. (B) The country-wise distribution in MFC research. The data is based on the number of articles mentioning MFC in the citation database Scopus in September' 2009.

only the integral composition of the bacterial community in the anode biofilm, but also the MFC performance including the power density (PD) and Coulombic efficiency (CE) (Chae et al., 2009). In this article, we have reviewed all the substrates that have been used in MFCs so far. Besides producing electricity, an objective of these systems is also to treat pollutants such as nitrates, sulfide and sulfates. Various pollutants that have been treated in these systems are also discussed in the forthcoming sections. The other main type of BES is the microbial electrolysis cell (MEC) which is a modified MFC for generating hydrogen from acetate and other fermentation end products by electrohydrogenesis. However, the substrates used in MECs are much lesser in variety than MFCs and therefore have not been discussed in this review.

2. Design and operation of MFCs

An appropriate design is an important feature in MFCs and researchers have come up with several designs of MFCs over the years with improved performance (Du et al., 2007). Fig. 2A and B

shows in detail the mode of operation and components of a typical two-chamber and a single-chamber MFC. In MFC, microorganisms oxidize organic matter in the anode chamber (anaerobic conditions) producing electrons and protons. Electrons transfer via the external circuit to the cathode chamber where electrons, protons and electron acceptor (mainly oxygen) combine to produce water (Li et al., 2009). In a two-chamber set up, the anode and cathode compartments are separated by an ion-selective membrane, allowing proton transfer from anode to cathode and preventing oxygen diffusion to the anode chamber. In the single-chamber MFC, the cathode is exposed directly to the air. Besides these two common designs, several adaptations have been made in MFC design and structure. These are discussed in detail by Du et al. (2007).

3. Substrates used in MFCs

In MFCs, substrate is regarded as one of the most important biological factors affecting electricity generation (Liu et al., 2009). A great variety of substrates can be used in MFCs for electricity

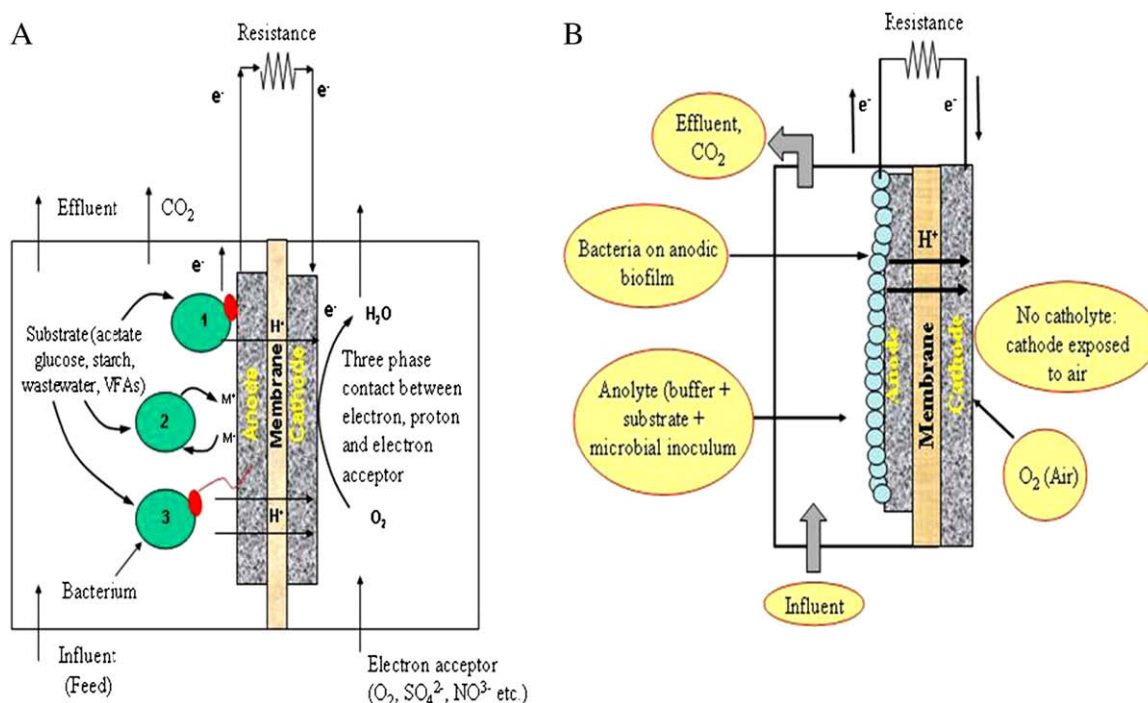


Fig. 2. (A) Simplified view of a two-chamber MFC with possible modes of electron transfer is shown. (1) Direct electron transfer (via outer membrane cytochromes); (2) electron transfer through mediators; and (3) electron transfer through nanowires. (B) Single-chamber MFC with open air cathode.

production ranging from pure compounds to complex mixtures of organic matter present in wastewater. So far the only objective of the various treatment processes is to remove pollutants from waste streams before their safe discharge to the environment. In the last century, activated sludge process (ASP) has been the mainstay of wastewater treatment. However, it is a very energy intensive process and according to an estimate, the amount of electricity needed to provide oxygen in ASPs in USA is equivalent to almost 2% of the total US electricity consumption (Electric Power Research Institute, 2002). At the same time, the addition of a second treatment step changes the status of several streams generated in the ASP treatment of agro-industry from “waste” to “raw material” which can eventually be utilized for the production of specific chemicals or energy (Kleerebezem and van Loosdrecht, 2007). Moreover, the emphasis of today’s waste management is on reuse and recovery of energy, which has led to new views on how these streams can be dealt with.

Table 1 presents a comprehensive list of substrates that have been used in MFC studies. It is difficult from literature to compare MFC performances, due to different operating conditions, surface area and type of electrodes and different microorganisms involved. Further, different researchers use different units to denote the performance of a MFC. One of the most common unit is current density, which is either represented as the current generated per unit area of the anode surface area (mA/cm^2) or current generated per unit volume of the cell (mA/m^3). In Table 1, for the sake of comparison, current density is mentioned in mA/cm^2 . In the articles where the current density was not expressed in mA/cm^2 , it was calculated based on the electrode dimensions and the current at maximum power reported. The information of electrodes and type of inoculum used is also mentioned. Some of the most common substrates and their influence on MFC performance are discussed below in detail.

3.1. Acetate

In most of the MFC studies so far, acetate has been the substrate of choice for electricity generation. The recalcitrance of many types

of wastewater makes them more difficult to be utilized as compared to acetate (Sun et al., 2009b). Acetate is a simple substrate and it is extensively used as carbon source to induce electroactive bacteria (Bond et al., 2002). In order to benchmark new MFC components, reactor designs or operational conditions, acetate is commonly used as a substrate because of its inertness towards alternative microbial conversions (fermentations and methanogenesis) at room temperature (Aelterman, 2009). Further, acetate is the end product of several metabolic pathways for higher order carbon sources (including the Entner–Doudoroff pathway for glucose metabolism) (Biffinger et al., 2008).

Using a single-chambered MFC, Liu et al. (2005) reported that the power generated with acetate (506 mW/m^2 , 800 mg/L) was up to 66% higher than that produced with butyrate (305 mW/m^2 , 1000 mg/L). Very recently, Chae et al. (2009) compared the performance of four different substrates in terms of CE and power output. Acetate-fed MFC showed the highest CE (72.3%), followed by butyrate (43.0%), propionate (36.0%) and glucose (15.0%). Also, when acetate was compared with a protein-rich wastewater as substrate in MFC, the MFC based on acetate-induced consortia achieved more than 2-fold maximum electric power, and one half of optimal external load resistance compared to the MFC based on consortia induced by a protein-rich wastewater (Liu et al., 2009). However, the protein-rich wastewater being a complex substrate provides the possibility of enriching more diverse microbial community than acetate. Having a more diverse microbial community helps to use various substrates or to convert complex organics to simpler compounds such as acetate which is used as electron donor for current production.

3.2. Glucose

Glucose is another commonly used substrate in MFCs. Kim et al. (2000) reported that the performance of a MFC containing *Proteus vulgaris* depended on the carbon source in the initial medium of the microorganism and glucose initiated cells in MFC run for a short time period compared with galactose. Rabaey et al. (2003) re-

Table 1
Different substrates used in microbial fuel cells (MFCs) and the maximum current produced.

Type of substrate	Concentration	Source inoculum	Type of MFC (with electrode surface area and/or cell volume)	Current density (mA/cm ²) at maximum power	Reference
Acetate	1 g/L	Pre-acclimated bacteria from MFC	Cube shaped one-chamber MFC with graphite fiber brush anode (7170 m ² /m ³ brush volume)	0.8	Logan et al. (2007)
Arabitol	1220 mg/L	Pre-acclimated bacteria from MFC	One-chamber air-cathode MFC (12 mL) with non-wet proofed carbon cloth as anode (2 cm ²) and wet proofed carbon cloth as cathode (7 cm ²)	0.68	Catal et al. (2008b)
Azo dye with glucose	300 mg/L	Mixture of aerobic and anaerobic sludge	One-chamber air-cathode MFC with carbon paper anode (36 cm ²)	0.09	Sun et al. (2009a)
Carboxymethyl cellulose (CMC)	1 g/L	Co-culture of <i>Clostridium cellulolyticum</i> and <i>G. sulfurreducens</i>	Two-chambered MFC with graphite plates as electrodes (16 cm ²) and ferricyanide catholyte	0.05	Ren et al. (2008)
Cellulose particles	4 g/L	Pure culture of <i>Enterobacter cloacae</i>	U-tube MFC with carbon cloth anode (1.13 cm ²) and carbon fibers as cathode	0.02	Rezaei et al. (2009b)
Corn stover biomass	1 g/L COD	Domestic wastewater	One-chamber membrane-less air-cathode MFC with carbon paper anode (7.1 cm ²) and carbon cloth cathode	0.15	Zuo et al. (2006)
Cysteine	385 mg/L	Sediment sample from 30 cm depth	Two-chambered MFC with carbon paper as electrodes (11.25 cm ²)	0.0186	Logan et al. (2005)
1,2-Dichloroethane	99 mg/L	Microbial consortia from acetate enriched MFC	Two-chambered MFC with graphite plate anode (20 cm ²) and graphite granules cathode	0.008	Pham et al. (2009a)
Ethanol	10 mM	Anaerobic sludge from wastewater plant	Two-chambered aqueous cathode MFC with carbon paper electrodes (22.5 cm ²)	0.025	Kim et al. (2007)
Farm manure	3 kg in water (20% w/v)	Self build up of anaerobic environment	One reactor vessel of manure with anode at the bottom and cathode above the manure; carbon cloth electrodes (256 cm ²)	0.004	Scott and Murano (2007)
Furfural	6.8 mM	Pre-acclimated bacteria from anode of a ferricyanide-cathode MFC	One-chamber air-cathode MFC with carbon paper anode and cathode (7 cm ²)	0.17	Luo et al. (2010)
Galactitol	1220 mg/L	Pre-acclimated bacteria from MFC	One-chamber air-cathode MFC (12 mL) with non-wet proofed carbon cloth as anode (2 cm ²) and wet proofed carbon cloth as cathode (7 cm ²)	0.78	Catal et al. (2008b)
Glucose	6.7 mM	Mixed bacterial culture maintained on sodium acetate for 1 year (<i>Rhodococcus</i> and <i>Paracoccus</i>)	One-chamber air-cathode MFC (12 mL) with non-wet proofed carbon cloth as anode (2 cm ²) and wet proofed carbon cloth as cathode (7 cm ²)	0.70	Catal et al. (2008a)
Glucuronic acid	6.7 mM	Mixed bacterial culture	One-chamber air-cathode MFC (12 mL) with non-wet proofed carbon cloth as anode (2 cm ²) and wet proofed carbon cloth as cathode (7 cm ²)	1.18	Catal et al. (2008a)
Lactate	18 mM	Pure culture of <i>S. oneidensis</i> MR-1	Two-chambered MFC with graphite felt electrode (20 cm ²)	0.005	Manohar and Mansfeld (2009)
Landfill leachate	6000 mg/L	Leachate and sludge	Two-chambered MFC with carbon veil electrode (30 cm ²)	0.0004	Greenman et al. (2009)
Macroalgae, <i>Ulva lactuca</i>	2500 mg/L COD	Primary clarifier overflow of wastewater plant	One-chamber air-cathode MFC (25 mL) with graphite brush anodes and platinized cathode	0.25	Velasquez-Orta et al. (2009)
Malt extract, yeast extract and glucose	1%	Pure culture of <i>E. cloacae</i>	Two-chambered salt bridge MFC with mediators and graphite plate as electrode (15 cm ²)	0.067	Mohan et al. (2008)
Mannitol	1220 mg/L	Pre-acclimated bacteria from MFC	One-chamber air-cathode MFC (12 mL) with non-wet proofed carbon cloth as anode (2 cm ²) and wet proofed carbon cloth as cathode (7 cm ²)	0.58	Catal et al. (2008b)
Microalga, <i>Chlorella vulgaris</i>	2500 mg/L COD	Primary clarifier overflow of wastewater plant	One-chamber air-cathode MFC (25 mL) with graphite brush anodes and platinized cathode	0.20	Velasquez-Orta et al. (2009)
Microcrystalline cellulose	7.5 g/L	Rumen microorganism from rumen of a cow	Two-chambered MFC with graphite plates as electrodes (84 cm ²)	0.02	Rismani-Yazdi et al. (2007)
Nitrilotriacetic acid (NTA)	48.5 mg/L	Oligotrophic consortium enriched with river water	Two-chambered MFC with graphite felt as electrodes (24 cm ²)	0.0005	Jang et al. (2006)
Phenol	400 mg/L	Mixed aerobic activated sludge and anaerobic sludge (1:1, v/v)	Two-chambered MFC with aqueous air cathode, carbon paper electrode (25 cm ²)	0.1	Luo et al. (2009)
Propionate	0.53 mM	Anaerobic sludge	Two-chambered MFC with carbon paper as electrodes (22.5 cm ²)	0.035	Oh and Logan (2005)
Ribitol	1220 mg/L	Pre-acclimated bacteria from MFC	One-chamber air-cathode MFC (12 mL) with non-wet proofed carbon cloth as anode (2 cm ²) and wet proofed carbon cloth as cathode (7 cm ²)	0.73	Catal et al. (2008b)
Sodium formate	20 mM	Anaerobic digested fluid from a sewage treatment plant	Two-chambered MFC with graphite felt as electrodes (4.5 cm ²)	0.22	Ha et al. (2008)

Table 1 (continued)

Type of substrate	Concentration	Source inoculum	Type of MFC (with electrode surface area and/or cell volume)	Current density (mA/cm ²) at maximum power	Reference
Sodium fumarate	25 mM	Pure culture of <i>G. sulfurreducens</i>	Stainless steel cathode (2.5 cm ²) half cells poised at –600 mV versus Ag/AgCl	2.05	Dumas et al. (2008)
Sorbitol	1220 mg/L	Pre-acclimated bacteria from MFC	One-chamber air-cathode MFC (12 mL) with non-wet proofed carbon cloth as anode (2 cm ²) and wet proofed carbon cloth as cathode (7 cm ²)	0.62	Catal et al. (2008b)
Starch	10 g/L	Pure culture of <i>Clostridium butyricum</i>	Two-chambered MFC with woven graphite anode (7 cm ²) and ferricyanide catholyte	1.3	Niessen et al. (2004)
Sucrose	2674 mg/L	Anaerobic sludge from septic tank	Two-chambered mediator-less MFC with stainless steel mesh as anode (213.29 cm ²) and cathode (176.45 cm ²); KMnO ₄ (0.2 g/L) as catholyte	0.19	Behera and Ghangrekar (2009)
Xylitol	1220 mg/L	Pre-acclimated bacteria from MFC	One-chamber air-cathode MFC (12 mL) with non-wet proofed carbon cloth as anode (2 cm ²) and wet proofed carbon cloth as cathode (7 cm ²)	0.71	Catal et al. (2008b)
Xylose	6.7 mM	Mixed bacterial culture	One-chamber air-cathode MFC (12 mL) with non-wet proofed carbon cloth as anode (2 cm ²) and wet proofed carbon cloth as cathode (7 cm ²)	0.74	Catal et al. (2008a)
Xylose and humic acid	10 mM	Domestic wastewater	Two-chambered MFC with plain carbon paper as electrode (76.5 cm ²)	0.06	Huang and Angelidaki (2008)
<i>Wastewaters</i>					
Artificial wastewater with glucose and glutamate	300 mg/L	Anaerobic sludge	Membrane-less MFC with anode (465 cm ²) at bottom and cathode (89 cm ²) at top of cylinder; graphite felt as both electrode	0.02	Jang et al. (2004)
Brewery wastewater	2240 mg/L	Full strength brewery wastewater	One-chamber air-cathode MFC with non-wet proofed carbon cloth as anode (7 cm ²) and wet proofed carbon cloth containing Pt as cathode	0.2	Feng et al. (2008)
Beer brewery wastewater	600 mg/L	Anaerobic mixed consortia	One-chamber air-cathode MFC with carbon fibers as anode	0.18	Wen et al. (2009)
Chocolate industry wastewater	1459 mg/L COD	Activated sludge	Two-chambered MFC with graphite rods as electrodes (16.485 cm ²) and ferricyanide as catholyte	0.302	Patil et al. (2009)
Domestic wastewater	600 mg/L	Anaerobic sludge	Two-chambered mediator-less MFC with plain graphite electrode (50 cm ²)	0.06	Wang et al. (2009a)
Food processing wastewater	1672 mg/L COD	Anaerobic sludge	Two-chambered MFC with carbon paper as electrodes (22.5 cm ²)	0.05	Oh and Logan (2005)
Meat processing wastewater	1420 mg/L	Domestic wastewater	One-chamber (28 mL) MFC with carbon paper electrodes (25 m ² /m ³)	0.115	Heilmann and Logan (2006)
Paper recycling wastewater	2.452 g/L	Diluted paper recycling wastewater	One-chamber MFC with graphite fiber-brush anode (5418 m ² /m ³ brush volume)	0.25	Huang and Logan (2008)
Protein-rich wastewater	1.75 g/L COD	Mesophilic anaerobic sludge	Two-chambered MFC with graphite rods as electrode (65 cm ²)	0.008	Liu et al. (2009)
Real urban wastewater	330 mg/L	Domestic wastewater	Separate anolyte (1000 cm ³) and catholyte chambers (100 cm ³) connected with a salt bridge; graphite cylinder anode (20 cm ²)	0.018	Rodrigo et al. (2007)
Starch processing wastewater	4852 mg/L COD	Starch processing wastewater	One-chamber air-cathode MFC with carbon paper anode (25 cm ²)	0.09	Lu et al. (2009)
Swine wastewater	8320 mg/L COD	Full-strength swine wastewater	One-chamber MFC (28 mL) with Toray carbon paper as anode (25 m ² /m ³) and carbon cloth as cathode	0.015	Min et al. (2005)
Synthetic acid-mine drainage water	0.007 M Fe ²⁺	Medium with NaCl and NaHCO ₃ sparged with N ₂ and CO ₂	Two-chambered MFC with carbon cloth anode (7 cm ²) and platinized carbon cloth cathode	0.064	Cheng et al. (2007)
Synthetic wastewater	12.1 g/L COD	Anaerobic mixed consortia producing hydrogen	Dual chamber MFC with graphite plate electrode (83.56 cm ²)	0.086	Venkata Mohan et al. (2008a)
Synthetic wastewater	16 g COD/day	Granular sludge from a upflow anaerobic sludge blanket (UASB) reactor	Membrane-less, mediator-less MFC with glassy carbon electrodes (160 cm ²)	0.017	Aldrovandi et al. (2009)
Synthetic wastewater	510 mg/L	Anaerobic culture from a pre-existing MFC	Dual chamber MFC with stainless tell as anode (170 cm ²) and graphite rods as cathode (150 cm ²)	0.008	Jadhav and Ghangrekar (2009)
Synthetic wastewater with molasses and urea	1000 mg/L	Anaerobic mixture from wastewater plant	Two-chambered MFC with copper wires as anode (20.1 cm ²) and gold covered copper wires as cathode	0.005	Kargi and Eker (2007)
Wastewater amended with acetate	1600 mg/L	Domestic wastewater	Submersible MFC, with an immersed anode (carbon paper, 16 cm ²) and an air-cathode chamber in an anaerobic reactor	0.08	Min and Angelidaki (2008)

ported that a maximum power density of 216 W/m³ was obtained from a glucose fed-batch MFC using 100 mM ferric cyanide as cathode oxidant.

Hu (2008) evaluated the feasibility of anaerobic sludge as fuel for electricity generation in MFC and compared it with glucose. In a baffle-chamber membrane-less MFC, anaerobic sludge added very limited substrate and a limited power (0.3 mW/m²) could be generated. However, with glucose in the same system, a maximum power of 161 mW/m² was generated. In another study, the energy conversion efficiency (ECE) of acetate and glucose as substrates in MFC was compared (Lee et al., 2008). The ECE was 42% with acetate, but was only 3% with glucose which led to a low current and power density as well. In a recent study by Chae et al. (2009), glucose-fed MFC generated the lowest CE as a result of electron loss by competing bacteria, but its relatively diverse bacterial structure enabled much wider substrate utilization and the greatest PD. The low CE was due to the fact that glucose is a fermentable substrate implying its consumption by diverse competing metabolisms such as fermentation and methanogenesis that cannot produce electricity. To explain the much wider substrate specificity of the glucose-enriched MFC than the others, Chae et al. (2009) proposed the presence of a more complex mixed consortium of diverse electricigens or their syntrophic bacteria as a result of the production of diverse fermentation byproducts during glucose degradation.

3.3. Lignocellulosic biomass

The abundance and renewability of lignocellulosic materials from agricultural residues renders them a promising feedstock for cost-effective energy production (Huang et al., 2008). However, lignocellulosic biomass cannot be directly utilized by microorganisms in MFCs for electricity generation. It has to be converted to monosaccharides or other low-molecular-weight compounds (Ren et al., 2007). Catal et al. (2008a) demonstrated that all monosaccharides that can be directly generated from hydrolysis of lignocellulosic biomass were good sources for electricity generation in MFCs. When cellulose is used as the substrate, electricity generation requires a microbial community with both cellulolytic and exoelectrogenic activities (Rezaei et al., 2009b).

Electricity generation in MFCs from corn stover waste biomass using samples prepared through either neutral or acid steam-exploded hydrolysis processes that convert the hemicellulose to soluble sugars was explored by Zuo et al. (2006). Maximum PDs using an air-cathode containing a diffusion layer and increased solution conductivity (20 mS/cm) were 371 mW/m² and 367 mW/m² for the neutral and acid hydrolysates (1000 mg COD/L, 250 Ω). Very recently, the use of raw corn stover as a substrate for electricity generation in a single-chambered MFC was demonstrated (Wang et al., 2009b) though the power output was much less than that with glucose as substrate.

No effective microorganisms for conversion of pentoses (one of the main components in lignocellulose hydrolysates) to bioethanol have been found yet, rendering a large fraction of residual plant material unsuitable for bioethanol production. Using xylose (typical pentose), PD of 69 mW/m² was reported at 10 mM concentration, which was less than PD for glucose (97 mW/m² at same concentration) indicating that xylose is more difficult to utilize for power generation than glucose (Huang and Angelidaki, 2008).

3.4. Synthetic wastewater

Synthetic or chemical wastewater with well-defined composition is also used by several researchers as it is easy to control in terms of loading strength, pH and conductivity. Venkata Mohan et al. (2008a,b) have used synthetic wastewater at different loading

rates in similar MFC configurations to achieve variable performances.

Several media used for bacterial growth contains significant amount of redox mediators, such as cysteine, and high strength wastewater contains reduced sulfur species, which can work as abiotic electron donor and increase power production for a short while (Aldrovandi et al., 2009) thus not representing the true performance of the system. This can be avoided by using a minimal salt medium with a single electron donor such as glucose or acetate. To check the influence of wastewater composition on the performance of MFC, Rodrigo et al. (2009) fed MFCs with two different synthetic wastewaters with the same organic pollutants (glucose and peptone) and same organic loading (315 mg/dm³) but with a different ratio of readily/slowly biodegradable substrate. The MFC fed with slowly biodegradable waste was more efficient in terms of electricity production probably due to the production of intermediates favoring electricity formation.

3.5. Brewery wastewater

Wastewater from breweries has been a favorite among researchers as a substrate in MFCs, primarily because of its low strength. Besides, it is suitable for electricity generation in MFCs due to the food-derived nature of the organic matter and the lack of high concentrations of inhibitory substances (for example, ammonia in animal wastewaters) (Feng et al., 2008). Although the concentration of brewery wastewater varies, it is typically in the range of 3000–5000 mg of COD/L which is approximately 10 times more concentrated than domestic wastewater (Vijayaraghavan et al., 2006). It could also be an ideal substrate for MFCs due to its nature of high carbohydrate content and low ammonium nitrogen concentration. Beer brewery wastewater treatment using air-cathode MFC was investigated by Feng et al. (2008) and a maximum PD of 528 mW/m² was achieved when 50 mM phosphate buffer was added to the wastewater. In this case the maximum power produced by brewery wastewater was lower than that achieved using domestic wastewater, when both wastewaters were compared at similar strengths. This might be due to difference in conductivities of two wastewaters. Diluting the brewery wastewater with deionized water decreased the solution conductivity from 3.23 mS/cm to 0.12 mS/cm. Recently, Wen et al. (2009) using a model based on polarization curve for the MFC, reported that the most important factors which influenced the performance of the MFC with brewery wastewater were reaction kinetic loss and mass transport loss (both were 0.248 V when current density was 1.79 A/m²). These can be avoided by increasing the concentration of brewery wastewater and by increasing the reaction temperature and using a rough electrode to provide for more reaction sites.

3.6. Starch processing wastewater

Starch processing wastewater (SPW) contains a relatively high content of carbohydrates (2300–3500 mg/L), sugars (0.65–1.18%), protein (0.12–0.15%) and starch (1500–2600 mg/L), representing an important energy-rich resource, which can be potentially converted to a wide variety of useful products (Jin et al., 1998). SPW was used as a fuel to enrich a microbial consortium generating electricity and current generation (0.044 mA/cm²) was coupled to a fall in COD from over 1700 mg/L to 50 mg/L in 6 weeks (Kim et al., 2004). Lu et al. (2009) operated a MFC with SPW containing 4900 mg/L of COD over four cycles and obtained a maximum voltage output and power density of 490.8 mV and 239.4 mW/m² in the third cycle. However, the CE was only 7%. They attributed this low CE to oxygen diffusion to the anode compartment resulting in

oxidization of other electron acceptors, biomass production and fermentation.

3.7. Dye wastewater

Azo dyes constitute the largest chemical class of synthetic dyes and are extensively present in effluent from dye-manufacturing industries and textile industries. Their removal from these effluents before discharge is of paramount importance as the intense color of these dyes leads to severe environmental problems such as obstruction of light and oxygen transfer into water bodies which in turn is detrimental to aquatic life (Pant et al., 2008). Besides, several of these dyes are also toxic in nature. Very recently, efforts have been made to utilize these dyes as a substrate in MFC leading to color removal from such dye-containing wastewaters as well as generating electricity. Sun et al. (2009a) reported accelerated decolorization of active brilliant red X-3B (ABRX3), a model azo dye, in a MFC when glucose and confectionary wastewater were used as co-substrates. Though higher dye concentrations (even up to 1500 mg/L) did not inhibit their decolorization; however, electricity generation from glucose was affected by higher concentrations of ABRX3 (>300 mg/L). This was attributed to the competition between azo dye and the anode for electrons from carbon sources. Thus, simultaneous treatment of azo dye-containing wastewater and readily biodegradable organic matter-containing wastewater could be achieved by mixing two kinds of wastewater in the MFCs, with the advantage of saving both cost and energy, however, the system still requires considerable improvements in terms of finding appropriate bacterial community that is capable of utilizing a mixture of dyes and other simple carbon sources in order to make MFCs a realistic solution for this kind of wastewater.

3.8. Landfill leachates

Landfill leachates are heavily polluted landfill effluents with a complex composition containing four major groups of pollutants: dissolved organic matter, inorganic macro-components, heavy metals, and xenobiotic organic compounds (Kjeldsen et al., 2002). The use of landfill effluent in a biological fuel cell for COD removal was first reported by Habermann and Pommer (1991), though no current production values were mentioned. An upflow air-cathode MFC generating electricity continuously from leachate for 50 h was reported by Zhang et al. (2008) with maximum volumetric power 12.8 W/m³ obtained at a current density of 41 A/m³. Recently, Greenman et al. (2009) demonstrated that it is possible to generate electricity and simultaneously treat landfill leachate in MFC columns. Gálvez et al. (2009) operated three MFCs fluidically connected in series for simultaneous leachate treatment and electricity generation.

3.9. Cellulose and chitin

Particulate substrates like cellulose and chitin are cheap and readily available biopolymeric materials which can be used for electricity generation. These renewable substrates also form a major component of organic matter in industrial and municipal wastewaters (Rezaei et al., 2009a). There have been only a few studies on use of particulate substrates in MFCs. For direct conversion of cellulose to electricity in MFC, the microorganism(s) must be able to hydrolyze cellulose anaerobically and be electrochemically active, utilizing anode as an electron acceptor while oxidizing metabolites of cellulose hydrolysis. PD up to 55 mW/m² using cellulose as the substrate and cattle rumen microorganisms as the catalyst have been reported (Rismani-Yazdi et al., 2007). Later, Ren et al. (2008) reported a power density of 153 mW/m² using carboxymethyl cellulose as substrate. Very recently, Rezaei et al.

(2009a) tested the effect of particle size on maximum power, power longevity and CE using different sized chitin particles. These authors reported that the maximum PD was lower for the largest (0.78 mm) particles (176 mW/m²), with the higher PD for the 0.28 mm (272 mW/m²) and 0.46 mm (252 mW/m²) particle sizes. Thus, using a solid substrate such as cellulose or chitin, the power production is limited due to a low rate of hydrolysis of the particulate material.

3.10. Sunlight

Solar energy can serve as an alternative energy source for MFC operation. Rosenbaum et al. (2005) proposed the concept of a 'living solar cell' in which the green alga *Chlamydomonas reinhardtii* produces hydrogen photosynthetically which in turn is oxidized *in situ* to produce current. Phototrophic MFCs represent an approach to convert solar energy into electric energy either through photosynthetic microorganisms (He et al., 2009) or living plants (Strik et al., 2008a). A solar powered MFC was described by Cho et al. (2008) in which only *Rhodobacter sphaeroides* (fed on succinate) was used as the anodic bacterium. The power output (790 mW/m²) in this case was dependent on both light and the nature of the nitrogen source. The plant MFCs in rice paddy fields have been reported to produce electricity by rhizosphere populations oxidizing organic carbon delivered to the rhizosphere (Kaku et al., 2008). Similar proof of principle was also demonstrated using Reed mannagrass (*Glyceria maxima*) and maximum power of 67 mW/m² anode surface was achieved (Strik et al., 2008a). Another type of phototrophic MFC, a photosynthetic algal MFC was investigated (Strik et al., 2008b) which produced a maximum power of 110 mW/m² surface area of photobioreactor. The organic matter produced in the algal photobioreactor via photosynthesis was supplied to a MFC for electricity generation.

3.11. Inorganic and other substrates

Apart from these above mentioned substrates, some other substrates have also been explored. Electricity generation with anodic sulfide oxidation was reported (Rabaey et al., 2006) with a PD of 39 mW/L. Huang and Logan (2008) reported the effectiveness of electricity production with paper recycling plant wastewater using MFC and obtained a maximum PD of 672 mW/m² after amending the wastewater with phosphate buffer. However, with unamended wastewater, the power output was only 144 mW/m² mainly due to low solution conductivity. Luo et al. (2009) reported the degradation of phenol and current generation in MFC. The power generation using phenol as the sole substrate was lower than that of glucose and the CE was less than 10% indicating a substantial loss. The large amount of wastewater produced in integrated biorefineries is also a potential source of energy (Kaparaju et al., 2009). Recently the use of MFCs to remove the fermentation inhibitors in cellulosic biorefineries including furfural, 5-hydroxymethylfurfural, vanillic acid, 4-hydroxybenzaldehyde and 4-hydroxyacetophenone while simultaneously producing electricity was demonstrated (Borole et al., 2009). A combination of a carbon monoxide (CO) fermenter and MFC as an anaerobic continuous process was also reported recently (Kim and Chang, 2009). The CO fermenter was enriched to produce acetate which was fed to a MFC to generate electricity. Though the conversion yield was quite low, it proved that syn-gas (mainly CO) can be converted to electricity through microbial process. 1,2-Dichloro ethane degradation by anodophilic bacteria enriched in MFCs was reported by Pham et al. (2009a). Further, removal of sulfate and thiosulfate in a single-chamber MFC inoculated with *Desulfovibrio desulfuricans* was investigated (Zhao et al., 2009) and a maximum current production of 0.115 mA/cm² was observed.

4. Current and power outputs achieved in MFCs using different substrates

The production of current in an MFC is directly linked to the ability of the bacteria to oxidize a substrate and transfer electrons resulting from this oxidation to the anode electrode. The current and PD, CE and pollutants removal efficiencies differ between the various studies according to the experimental conditions (initial wastewater composition, concentration, and MFC set up conditions). Table 1 presents the current density (mA/cm²) at maximum power density (W/m²) achieved using various substrates in MFCs. With similar designs of MFC, 506 mW/m² was produced with acetate (Liu et al., 2005), but 261 mW/m² with swine wastewater (Min et al., 2005) and 146 mW/m² with domestic wastewater (Liu and Logan, 2004). The maximum power density produced appears to be related to the complexity of the substrate (i.e. single compound versus several compounds). Heilmann and Logan (2006) reported that with substrates like peptone and meat processing wastewater containing many different amino acids and proteins, lower power was produced than achieved using single compound like bovine serum albumin (BSA). The power generation measured using xylose as substrate was lower than studies with other fuels such as acetate or glucose (Huang et al., 2008). However, the fact that xylose bioconversion in MFCs takes place at room temperature and relatively low substrate concentration levels, whereas anaerobic digestion generally fails due to low reaction rates, may make the MFC a complementary technology to the anaerobic digestion for celluloses and its hydrolytes (Pham et al., 2006). Recently, while evaluating the potential of various eco-systems in harnessing bioelectricity through benthic fuel cells, Venkata Mohan et al. (2009) reported that the substrate concentration of the water body showed significant influence on the power generation as they act as carbon source (electron donor) for the benthic metabolic activity. Water bodies containing higher organic matter were able to generate higher power output.

The beginning 10 years of research on MFCs have resulted in a 10,000-fold increase in the current density obtained from MFCs (Rabaey et al., 2004). This has further improved in recent years. Nevin et al. (2008) reported that *G. sulfurreducens* grown on acetate produced 2.15 kW/m³ anode volume, which is the highest MFC power density reported to date. Similarly, a new axenic strain *Rhodospseudomonas palustris* DX-1, isolated from an MFC produced higher power output (2720 mW/m²) than other mixed cultures (Xing et al., 2008). However, at present the power generated by MFCs is low from the view of large-scale wastewater treatment. In fact the only MFC type that has been used for practical applications is sediment MFCs which harvest power from sediment by embedding an anode in sediment and connecting it via an electrical circuit to a cathode placed in the overlying aerobic seawater, making it feasible to power on-site to sensors and telemetry devices in remote oceanic areas (Tender et al., 2008). It is expected that with time, given the continued interest and support for this research, the output will reach a usable level for other applications as well.

5. The current challenges faced by these systems and the way ahead

Despite the fact that in recent years the power generation from MFCs have improved considerably and also reached the level of primary power target at least in small lab-scale systems, the scale-up is still a big challenge. Moreover, the high cost of cation exchange membranes, the potential for biofouling and associated high internal resistance restrain the power generation and limit the practical application of MFCs (Hu, 2008). In case of phototroph-

ic MFCs, the need for artificial illumination (Strik et al., 2008b; He et al., 2009) exerts extra energy input for the system and raises the cost. Domestic wastewater, which has organic matter with embedded energy content, contains almost 10 times the energy needed to treat it (WERF, 2009). While emerging technologies are promising, none of the processes available today can yet fully extract all the energy available in wastewater without further investment in their research and development. The actual performance of BESs with reactor volumes larger than 1 L is still lower than the goal of 1 kW/m³, which is considered as the threshold for feasible industrial application for energy recovery from organic matter (Pham et al., 2009b). A major drawback associated with MFCs is the start-up time which may vary from 4 to 103 days depending on the inoculum, electrode materials, reactor design, operating conditions (temperature, external loading, etc.) but most importantly on the substrate being fed into the system (Wang et al., 2009a). Another significant impediment in scaling up of MFCs for wastewater treatment is the lack of buffer capacity of electrolytes (You et al., 2009).

There are several ways by which the existing limitations in MFCs could be overcome. It is agreed that the power output of most MFCs is too low for any envisioned applications (Lovley, 2008). Besides, the high cost of a precious metal catalyst such as platinum which is usually needed on a cathode is also a big hindrance in up-scaling of these systems. Open air biocathodes proposed by Clauwaert et al. (2007b) could be a possible solution in future. The replacement of platinized cathodes with non-platinized ones with a similar efficiency is a major improvement in this area (Van Bogaert et al., 2009; Zhang et al., 2009a). The use of manganese dioxide as an alternative cathode catalyst in MFCs (Zhang et al., 2009b) and stainless steel and nickel alloys in MECs (Selembu et al., 2009) has also been suggested. Besides the substrates mentioned in this article, there are several other possible substrates which can be tried in these systems. For example, the wastewater from canning of fruits and vegetables (with a COD ranging between 1000 and 10,000 mg/L); whey, the major waste product from dairy industry (with COD from 60,000 to 80,000 mg/L); waste and wastewater from livestock industry particularly slaughterhouses are all potential substrates for MFC. The effluent from cane-molasses based distilleries, which is highly rich in organic load and produced in enormous volumes (Pant and Adholeya, 2007) could also be a potential substrate for MFCs.

Lovley (2009) suggested that despite the present slow rate of substrate conversion to electricity in BES, there are several other potential applications for microbe-electrode technology. This may be in the form of implanted medical devices using blood sugar as fuel, microbial transistors, circuits and electronic computing devices. Reactions at the bioanode can be directed towards the production of valuable compounds from inexpensive substrates (Pham et al., 2009b). The organic matter in waste streams can be used as the substrate for anodic microorganisms for the production of polyhydroxybutyrate (PHB) (Freiguia et al., 2007). Carbon dioxide capture and conversion to useful compounds in a MFC is another lucrative application, that has partly been realized recently (Cao et al., 2009). These authors reported the possibility of direct electron transfer between a cathode and microorganisms for fixation of CO₂ in biomass.

MFCs have also been used for treatment of recalcitrant compounds at the bioanode or cathode side. When contaminants serve as electron acceptors in the MFC cathode chamber, the environmental benefits of MFCs could be greatly enhanced. Denitrifying MFCs in which microorganisms in the cathode performed a complete denitrification by using electrons supplied by microorganisms oxidizing acetate or glucose in the anode have been reported (Clauwaert et al., 2007a; Jia et al., 2008). Another mani-

festation of these systems is for the production of certain chemical compounds. Recently, the H₂O₂ production at a carbon felt cathode using the electricity generated by a MFC was demonstrated by [Zhu and Ni \(2009\)](#). In the same system, *p*-nitrophenol was also degraded completely with a maximum power output of 143 mW/m². Yet another application of these systems could be as biosensors for wastewaters ([Kim et al., 2003](#)). [Di Lorenzo et al. \(2009\)](#) reported a biochemical oxygen demand (BOD) biosensor based on single-chamber MFC with air cathode and running on artificial wastewater and obtained a good correlation between COD concentration and current output.

6. Conclusion and future perspectives

This review summarizes the various substrates that have been used in MFCs for current production as well as waste treatment. Yet, the list is by no means exhaustive as newer substrates are brought under these systems with improved outputs both in terms of power generation as well as waste treatment. In the initial years, simple substrates like acetate and glucose were commonly used, but in recent years researchers are using more unconventional substrates with an aim of utilizing waste biomass or treating wastewater on one hand and improving MFC output on the other. Bioenergy in the form of electricity from renewable and waste biomass through MFCs have great development potential both in terms of energy self sufficiency as well as reducing competition with food production as is the apprehension with conventional biofuels. It is hoped that in coming years, with the expected improvement in this technology and lower costs, more variety of substrates will be used leading to a sustainable and economical bioenergy. These improved systems will be able to produce energy (electricity/hydrogen) from almost any renewable material including wastes and plant based biomass. The key conclusions that can be drawn are:

1. Substrates being used in both MFCs have grown in complexity and strength (higher organic loading rate). A complex substrate helps in establishing a diverse and electrochemically active microbial community in the system while a simple substrate is easier to degrade and improves the electric and hydrogen output of the system.
2. The output of these systems (electric current and electric power) is still some way from large-scale applications. More technological advancements in terms of material, costs and substrates being used are necessary to bring these systems at a level where they can be commercially exploited.
3. Several new substrates hitherto exploited can be brought as substrates under the MFC set ups. These may include the wastewaters from molasses based distilleries rich in organic matter and produced in large volumes, wastewater from large number of biorefineries, wastewaters from pharmaceutical industry with recalcitrant pollutants, waste plant biomass (agriculture residue) which is burnt at this moment, etc.
4. The integration of MFCs with existing separation, conversion and treatment technologies is probably the best option wherein the effluent from one stream can be used as a feed for the other one.

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