Simultaneous sewage treatment and electricity generation in membrane-less microbial fuel cell

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ABSTRACT

Long term performance of mediator-less and membrane-less microbial fuel cell (ML-MFC) was evaluated for treatment of synthetic and actual sewage and electricity harvesting. The anode chamber of ML-MFC was inoculated with pre-heated mixed anaerobic sludge collected from a septic tank. The ML-MFC was operated by feeding synthetic wastewater for first 244 days, under different organic loading rates, and later with actual sewage for next 30 days. Maximum chemical oxygen demand (COD) removal efficiency of 91.4% and 82.7% was achieved while treating synthetic wastewater and actual sewage, respectively. Maximum current of 0.33 mA and 0.17 mA was produced during synthetic and actual sewage treatment, respectively. Maximum power density of 6.73 mW/m^2 (13.65 mW/m^3) and maximum current density of 70.74 mA/m^2 was obtained in this membrane-less MFC with successful organic matter removal from wastewater.

Key words | electricity recovery, membrane-less microbial fuel cell, sewage treatment

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INTRODUCTION

Due to global environmental concerns and energy insecurity, there is emergent interest to find out cost effective wastewater treatment process and sustainable clean energy source, without use of fossil fuel. A microbial fuel cell (MFC) has a great potential to offer solution to this problem by generating direct electricity during oxidation of organic matter. MFCs have recently received increased attention as a means to produce 'green' energy from organic wastewater or synthetically prepared carbohydrate substrates. MFC consists of anode and cathode chambers separated by a proton exchange membrane (PEM). The organic matter gets oxidized by bacterial action in the anode chamber, producing electrons and protons. The electrons are passed to an anode by either action of mediators or through electrochemically active bacterial cells, enriched in the MFC. These electrons are transferred through external circuit to the cathode and protons are transferred internally through PEM. Thus, current is produced, to a cathode where electrons and protons are combined with oxidant supplied externally in cathode. Since, organic matter present in the wastewater can act as a fuel, MFCs show promise as a wastewater treatment process and simultaneously produce electricity (Liu *et al.* 2004). Excess sludge production in MFC is very low as compared to conventional aerobic processes (Rabaey *et al.* 2003), which will help in minimizing overall operating cost of the treatment plant by decreasing the cost of further sludge management.

Due to the non-conducting nature of most of the microbial cell surface structures involved in fermentation, electron transport to the electrode could not be efficient. Electrochemical mediators are generally employed to render efficient electron transfer from the microbial cells to the electrode. The mediators are usually expensive and can be toxic to the microorganisms, hence may limit the long-term commercial application of mediated MFCs for wastewater treatment (Bond & Lovley 2003). Recently, numbers of bacteria have been isolated based on their ability to transfer electrons to the electrode (Du *et al.* 2007). Such microbial communities are referred as adapted anodophilic consortia (Angenent *et al.* 2004). Mediators

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are not required in this MFC, for electron transfer to electrode, and it is referred as mediator-less MFC. Mediator-less MFCs are expected to have more commercial application potential. In addition to the microorganisms that can transfer electrons to the anode, the presence of other organisms appears to benefit MFC performance. MFCs operated using mixed cultures gave substantially greater power densities than those with pure cultures (Rabaey *et al.* 2004). Mixed cultures are more suitable for wastewater treatment, as single organisms metabolize quite limited organic compounds (Kim *et al.* 2007).

Proton transfer through the membrane can be a rate limiting factor, especially with fouling expected due to presence of suspended solids and soluble contaminants in a large scale wastewater treatment process. Membranes are expensive and hence may limit acceptance of MFC on a large scale wastewater treatment (Ghangrekar & Shinde 2007). It has recently been demonstrated that removing the PEM from an MFC can increase power density by a factor of 1.9 for glucose and 5.2 for wastewater (Liu & Logan 2004). Part of this increase in power was attributed to an enhancement of the proton flux from the anode to the cathode. A membrane-less microbial fuel cell (ML-MFC) was developed and used successfully by Jang *et al.* (2004).

If wastewater treatment efficiency and electricity recovery from the ML-MFC could be improved, this device has a greater potential for commercial application. Operational aspects of this ML-MFC need further attention to make its performance comparable with MFC using PEM and established anaerobic process, such as UASB reactor. It will be useful to examine the optimum organic loading rate (OLR) achieved by MFCs and compare it with established treatment systems (Logan *et al.* 2006). MFCs still need a significant breakthrough to become economically competitive. Eliminating use of membrane may help in drastically reducing production and operating cost of this device. However, their overall applicability as a wastewater treatment and electricity harvesting process is still largely unexplored (Rabaey & Verstraete 2005). Performance of MFC in long term wastewater treatment needs further attention, particularly with respect to defining optimum environmental condition which will support growth and sustenance of anodophilic microorganism. The objectives of this work were: 1) to evaluate long term performance of membrane-less and mediator-less microbial fuel cell, using simple graphite electrodes, for synthetic and actual sewage treatment at ambient temperature under different OLRs; and ii) to explore possibility of using mixed anaerobic sludge as inoculum for enriching anodophilic microorganisms.

MATERIALS AND METHODS

Membrane-less microbial fuel cell

The membrane-less microbial fuel cell used in this study was made of acrylic cylinder having internal diameter of 10 cm and total working volume of 4.6 L (Figure 1). The anode chamber was placed at the bottom, and the cathode chamber was at the top of cylinder-shaped reactor. Glass wool (4 cm depth) and glass bead (4 cm depth) were placed at the upper portion of the anode chamber. Solid graphite rod was used for both anode and cathode, separated by distance of 20 cm. Apparent surface area of each anode and cathode was 46.65 cm^2. Wastewater was supplied at the bottom of the anode chamber and the effluent left through cathode chamber at the top. Electrodes were connected with copper wire through a variable resistor.

Inoculation and operation

The ML-MFC was inoculated with anaerobic sludge (volume 1.0 L) collected from septic tank, and operated for first 15 days without any pretreatment to the inoculum. After 15 days, sludge from the anode chamber was removed, heat-treated at 100°C for 15 minutes to suppress the

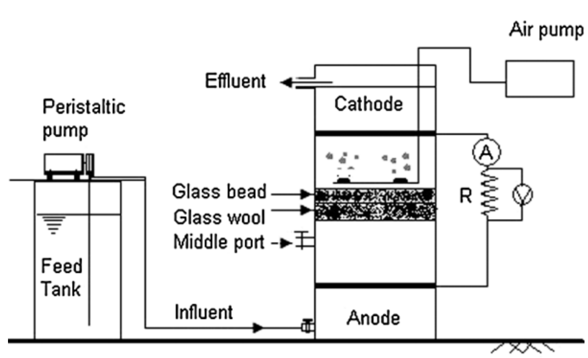

Figure 1 | Membrane-less microbial fuel cell used in the study.

methanogens, cooled to room temperature, and re-inoculated in the anode chamber. No inoculum was added in cathode chamber. Synthetic wastewater, containing sucrose as a carbon source, was used as a feed for initial 244 days as per the composition provided by Ghangrekar & Shinde (2007). Influent pH was maintained in the range of 7.2 to 7.6. Performance of the ML-MFC was evaluated under different OLRs using synthetic wastewater. Later for next 30 days, the performance was evaluated for treatment of sewage. Settled sewage was brought daily from the sewage pumping station of IIT Kharagpur for feeding. Cathode chamber was aerated at rate of 60 ml/minute in all the experiments. All experiments were conducted at room temperature ranging from 27 to 33°C.

Analyses

Influent and effluent samples were analyzed for chemical oxygen demand (COD), pH, dissolve oxygen (DO), alkalinity, total dissolved solids (TDS) daily and for biochemical oxygen demand (BOD), ammonia nitrogen, total Kjeldahl nitrogen (TKN), and most probable number (MPN) once in a week as per the *Standard Methods* (APHA 1998). COD was determined for the settled wastewater sample. Suspended solids (SS) and volatile suspended solids (VSS) in the effluent and for sludge were determined as per the APHA (1998) twice a week. The microstructure of the biofilm developed on the electrodes and granules developed in the anode chamber were examined using scanning electron microscopy (SEM; Model JSM-5800, JOEL, Japan). The potential and current were measured using a digital multimeter daily and converted to power according to $P = iV$ where $P =$ power (W), $i =$ current (A), and $V =$ voltage (V).

RESULTS AND DISCUSSION

Performance of ML-MFC during start-up

After inoculation, for first four days ML-MFC was operated under batch mode at HRT of 24 h. From fifth day onwards it was operated in a continuous mode at a feeding rate of 5.01 L/d. The electric current production reached to 0.3 mA on fifth day of continuous operation and gradually decreased to 0.1 mA on 14th day. The methanogens growth in the anode chamber with time might have reduced the availability of electron to the anode, and reduced the current. The operation of the ML-MFC was stopped and the sludge was removed, heat treated and re-inoculated in anode chamber with total volume of 1.0 L having SS and VSS of 117.9 g/l and 32 g/l, respectively. Slow increase in current for the first week and a rapid increase in the next week was observed. Stable conditions regarding COD removal and current production were observed afterwards.

The ML-MFC was operated at four different external resistances during initial enrichment process for 55 days. After 55 days of operation the average COD and BOD removal efficiencies were 87.53% and 90.67%, respectively. The corresponding OLR with respect to anode chamber was 0.78 kg COD/m^3.d and specific loading rate was 0.12 kg COD/kg VSS.d. At steady state, the effluent COD and BOD concentrations were 43 mg/l and 25 mg/l, respectively (Table 1). The COD removal efficiency obtained in the ML-MFC is in agreement with reported values, in the range of 80 to 90% (Jang *et al.* 2004; Liu *et al.* 2004; Oh & Logan 2005). Rather the overall efficiency observed was on higher side of the range, and demonstrated ability of ML-MFC to be used as the effective wastewater treatment process. However, the COD removal efficiency of anode chamber was only 43.85%. Contribution of anode chamber to overall COD removal seems to be lower in this ML-MFC. In a single chamber MFC, COD removal efficiency of 80% is reported at HRT of 33 h by Liu *et al.* (2004). Thus, further investigations are necessary to enhance organic matter conversion in anode, as reported for single chamber MFC. Enhancing anode efficiency will improve overall performance of ML-MFC for organic matter removal and electricity production.

COD and BOD removal under different OLR in ML-MFC

The ML-MFC was operated at different OLRs and influent COD concentrations to study the effect of OLR on the performance using a synthetic wastewater and actual sewage (Table 1). For influent COD around 300 mg/l, maximum average COD removal efficiency of 89.37% was observed, when OLR was 1.32 kg COD/m^3.d. At a higher COD concentration of 608 mg/l, corresponding to OLR of 2.65 kg COD/m^3.d, removal efficiency was 91.44%; and the effluent

Table 1 | Performance of the ML-MFC at different organic loading rates

			COD (mg/l)			BOD (mg/l)			Effluent DO (mg/l)
Days	OLR (kg COD/m^3.d)	HRT (h)	Inlet	Middle	Outlet	Inlet	Middle	Outlet	
0–15	0.72 (±0.09)	22.03	329 (±45)	249 (±19)	163 (±24)	ND	ND	ND	ND
16–35	0.87 (±0.07)	22.03	400 (±34)	312 (±23)	72 (±21)	280*	ND	48*	4.33 (±0.3)
36–55	0.68 (±0.04)	22.03	312 (±17)	208 (±55)	56 (±11)	240*	ND	35*	3.75 (±0.4)
56–121	0.78 (±0.08)	22.03	359 (±70)	201 (±36)	43 (±10)	268 (±18)	162(±13)	25 (±5)	4.14 (±0.4)
122–154	1.32 (±0.03)	11.02	299 (±14)	135 (±8)	32 (±3)	219 (±8)	102(±11)	18 (±1)	4.98 (±0.2)
155–189	2.00 (±0.08)	7.35	307 (±25)	167 (±12)	50 (±6)	248 (±9)	129 (±4)	27 (±2)	4.9 (±0.2)
190–244	2.65 (±0.05)	11.02	608 (±24)	302 (±21)	52 (±8)	461 (±11)	213(±21)	21 (±2)	4.88 (±0.2)
245–274†	0.75 (±0.04)	11.02	173 (±17)	87 (±10)	30 (±8)	117 (±8)	60 (±13)	21 (±3)	4.84 (±0.2)

ND-Not Determined.
* Average of two samples.
OLR- with respect to anode chamber.
†Actual sewage was used.
COD middle - COD at outlet of anode chamber.
Values in parentheses indicate standard deviation.

COD and BOD were 52 mg/l and 21 mg/l, respectively. This higher efficiency might be due to increased biomass concentration in anode and cathode chamber. When COD was increased from 300 to 600 mg/l, contribution of anode chamber for COD removal was 50.30%. Removal of remaining organic matter in cathode chamber showed higher growth of aerobic biomass in this chamber.

For COD concentration of wastewater around 300 mg/l, the performance was optimum at HRT of 11.02 h. At similar HRT, when COD concentration was increased to 608 mg/l, the ML-MFC showed higher COD removal. For higher and lower HRT, decrease in COD removal efficiency of anode chamber was observed. The effect of HRT on performance of ML-MFC is prominent. At HRT of 7.35 h decrease in overall COD removal was observed as compared to HRT of 11.02 h. Although, application of very low HRT (3 h) is reported, reduction in COD removal efficiency with decrease in HRT is reported (Liu *et al.* 2004). Overall, this device has demonstrated successful treatment of low strength wastewater under the OLR range normally used in anaerobic processes. Further studies are required to explore the maximum loading capacity.

Power production observed in ML-MFC

Power production during initial start-up

After inoculation with preheated sludge and application of feed in continuous mode, slow increase in current was observed in the ML-MFC with duration of operation, similar to the observation reported by Jang *et al.* (2004). ML-MFC took two weeks to get acclimatized with necessary microbial culture development to produce stable current. During start-4 up, maximum current of 0.175 mA was observed with closed circuit voltage of 0.19 V (Table 2). The open circuit voltage (OCV) was 0.3 V after fifteen days of operation with maximum power density of 6.73 mW/m^2 and current density of 37.51 mA/m^2 (Table 2). The power density and current density observed in this ML-MFC, using simple graphite electrode, were higher than the reported value of 1.3 mW/m^2 and 9 mA/m^2, respectively (Jang *et al.* 2004).

Power production under different OLRs

From the electricity generation observed in ML-MFC under different OLRs (Table 2) it can be said that, there is no correlation between OLR and the power and current produced. The power and current produced were mainly dependent on the HRT used. As HRT was decreased from 11.02 h to 7.35 h (corresponding to OLR of 1.32 kg COD/m^3.d and 2.0 kg COD/m^3.d, respectively), power density decreased from 1.22 mW/m^2 to 0.39 mW/m^2. Thus, indicating that, higher HRT is favourable for obtaining higher power density. At similar influent COD concentration (about 300 mg/l), the current density was maximum

Table 2 | Power production observed in the ML-MFC

Days	OLR (kg COD /m^3.d)	Current (mA)	Voltage (mV)	Resistance (Ω)	Power density (mW/m^2)	Current Density (mA/m^2)
00–15	0.72 (±0.09)	0.091 (±0.011)	116 (±7)	100	2.29 (±0.39)	19.50 (±2.35)
16–35	0.87 (±0.07)	0.175 (±0.007)	188 (±3)	10	6.73 (±0.44)	37.51 (±1.5)
36-55	0.68 (±0.04)	0.148 (±0.008)	175 (±7)	25	5.46 (±0.39)	31.72 (±1.71)
56–121	0.78 (±0.08)	0.121 (±0.01)	151 (±5)	50	3.96 (±0.46)	25.94 (±2.14)
122–154	1.32 (±0.03)	0.32 (±0.01)	17.45 (±1.30)	50	1.22 (±0.13)	68.59 (±2.14)
155–189	2.0 (±0.08)	0.17 (±0.01)	10.62 (±1.39)	50	0.39 (±0.067)	36.44 (±2.14)
190–244	2.65 (±0.05)	0.33 (±0.02)	17.84 (±1.01)	50	1.29 (±0.16)	70.74 (±4.28)
245–274*	0.75 (±0.04)	0.17 (±0.02)	9.45 (±1.01)	50	0.35 (±0.08)	36.45 (±4.28)

OLR-with respect to anode.
* Actual sewage was used as substrate.

at HRT of 11.02 h. For lower and higher HRT, the current density decreased. For similar COD concentration higher HRT favoured more power density; whereas, there is an optimum range of HRT for getting maximum current density.

Keeping HRT at 11.02 h and increasing COD concentration from 299 mg/l to 608 mg/l, (OLR 1.32 kg COD/m^3.d and 2.65 kg COD/m^3.d, respectively) change in current production and power density was very less. Thus, even by doubling the organic load the power output could not be enhanced, due to lower coulombic efficiency at higher loading rates. This shows the limitation of operating parameters in MFCs. This might be due to the faster growth of the fermentative bacteria as compared to the electrochemically active bacteria acidifying the anode at this loading rate (Rabaey *et al.* 2003). Although, operation of MFC at OLR higher than 2.65 kg COD/m^3.d is reported in literature, this ML-MFC performance showed reduction in coulombic efficiency with increase in OLR. Power density and current density could not improve with increase in OLR, although successful wastewater treatment could be achieved at higher OLR.

In the present experiment, preheated septic tank sludge was used as inoculum instead of any special culture without any external mediator addition in anode chamber. Although, the voltage observed across the resistance was less, the power density and current density observed are in agreement with the values reported for some MFCs using membrane and specialized culture (Rabaey & Verstraete 2005). The voltage observed across the resistance was much less than the open circuit, probably due to open joints between the electrodes and wire. From these observations it can be said that, production of electricity from ML-MFC is possible, by using preheated anaerobic sludge as inoculum, and it can become an attractive option to recover power during wastewater treatment to compensate the cost of treatment. Maximum power production reported in the literature varies with type of fuel cell, type and concentration of substrate, mediators and culture of biomass used as inoculum. Using high COD strength wastewater, higher power density than obtained in the present experiment is reported (Liu *et al.* 2004; Oh & Logan 2005; Kim *et al.* 2007). These MFCs are either having PEM, and/or using specially cultured anodophilic microbes for efficient electron transfer, or using of complex electrodes, where higher power output is expected. The current density of 70.74 mA/m^2 obtained at HRT of 11.02 h is in agreement with the reported values.

Since the COD removal efficiency in the anode chamber was less than 50%, remaining COD removal occurred in the cathode chamber. Aerobic biomass built-up was observed in the cathode chamber with time. It was noticed that with high concentration of aerobic biomass in the aerated cathode chamber, the power output was reducing. After removing the biomass, improvement in the power output was noticed. Less power density could be attributed to aerobic biomass growth and its intervention by following aerobic respiratory oxidation of organic matter. Thus, electrons and protons might have been limited for expected cathode reaction, and they have been consumed in high amount for usual aerobic oxidation, hence reducing coulombic efficiency of ML-MFC. To increase power output, modifications in the ML-MFC design and operational strategy are required to enhance oxidation of organic matter in anode chamber and reduce biomass growth and accumulation in cathode chamber. Efforts are also necessary to develop a protocol for enriching anodophilic microbes and suppressing growth of methanogens from the mixed culture inoculum. This can be done by applying stress conditions either by high temperature, desiccation and drying, nutrient limitation, adverse chemicals addition, organic solvent, and radiation (Cheong & Hansen 2006). If power generation in ML-MFC could be increased, due to its lower production and operating cost it may provide a new method for sustainable wastewater treatment with simultaneous electricity generation, to offset cost of treatment.

Performance ML-MFC treating actual sewage

After 244 days of operation with synthetic feed, the ML-MFC was fed with actual sewage and operated at HRT of 11.02 h (Table 1). Stable electricity production was observed after nine days, due to change of substrate. The average COD removal efficiency of 82.7% was observed (Figure 2) producing effluent with COD and BOD values of 30 and 21 mg/l, respectively. BOD and COD removal in the anode chamber was about 50%. MFC treating actual sewage is reported to give COD removal up to 80% at HRT of 33 h (Liu *et al.* 2004). The observed COD and BOD removal efficiency in this experiment are well in agreement with the reported value. This demonstrated the ability of the ML-MFC to treat sewage giving more than 80 percent COD and BOD removal, even at the short HRT of 11.02 h, and by using pre-heated septic tank sludge as inoculum. The current production of 0.17 mA (Table 2) was observed with power density varying in the range of 0.26 to 0.49 mW/m^2.

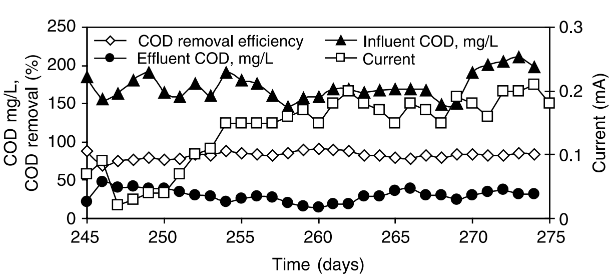

Figure 2 | Performance of ML-MFC during actual sewage treatment.

Variation in the current production was observed due to variation in the influent COD concentration, and the current production was varied in the range 0.15 to 0.2 mA.

The raw sewage used as the feed had pH in the range of 7.0 to 7.4, and the effluent of ML-MFC had pH in the range of 7.4 to 8.2. Alkalinity and TDS in the raw sewage were in the range of 142 to 268 mg/l as $CaCO_3$ (average 221 mg/l) and 228 to 320 mg/l (average 279 mg/l), respectively. The effluent alkalinity of 100 to 220 mg/l and the effluent TDS of 175 to 280 mg/l was observed. The SS concentration in the sewage, used as feed, was ranging between 50 and 150 mg/l and average effluent SS concentration was 20.1 mg/l. The TKN removal efficiency of 59.8% and ammonia nitrogen removal of 59.0% was observed. MPN was analyzed in the influent and effluent thrice during actual sewage treatment. Raw sewage used as feed showed MPN/100 ml in the range of 9×10^6 to1.6×10^7. The corresponding MPN values for effluent of ML-MFC were 1.7×10^4 and 8×10^4. This showed that ML-MFC has the ability to reduce MPN by 2 to 3 log-scale. So far the MPN reduction in the MFCs is not reported in the literature. Further studies are required to explore the mechanism of MPN removal and to find out contribution from each chamber for this removal.

Granulation in biomass and biofilm formation on the electrodes

In the ML-MFC after 35 days of operation granules formation was visible in the anode chamber. Sludge sample was collected on 50th day for microscopic examination from anode chamber. The sludge was granulated partially having 1 to 2 mm size of the granules with typical dark gray colour. The SEM image of a typical granule (Figure 3A) showed that the granule had multiple cracks on the surface,

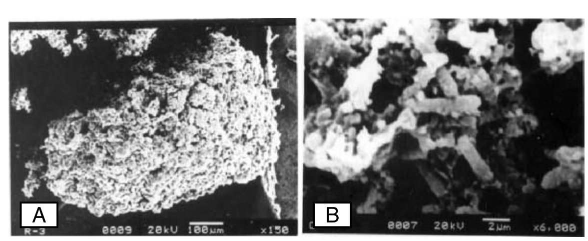

Figure 3 | SEM images (A) typical granule observed in anode chamber, and (B) anode biofilm collected on 274th day showing short rod shaped bacteria.

with highly porous inner structure. The granules were comprised of similar short rod shaped bacteria as observed on the anode (Figure 3B) along with other long rod shaped bacteria. These granules, unlike those of methanogenic USAB granules, did not exhibit a layered structure. The biofilm formed on the electrodes was scrapped on 90th day and 274th day for SEM. A close examination revealed that there were two predominant bacterial morphologies. On the anode electrode small rod shape (3–5 μm in the length and 1–2 μm in width) were dominating (Figure 3B), resembling to family of *Geobacteraceae* (Bond *et al.* 2002). On the cathode, rod shape bacteria (greater than 50 μm in the length and 5–7 μm in the width) were in dominance (figure not shown) along with other coccal-type bacteria.

CONCLUSIONS

The membrane-less MFC inoculated with pre-heated mixed anaerobic sludge showed excellent capability to treat synthetic wastewater and actual sewage. Under different OLR and using synthetic wastewater as feed, the maximum COD removal efficiency of 91.4% was obtained at OLR of 2.65 kg COD/m^3.d. For actual sewage treatment the efficiency was 82.7%. The ML-MFC also demonstrated capability to remove nitrogen and to reduce MPN from the sewage. Under different operating conditions, the ML-MFC produced maximum power density of 6.73 mW/m^2 and current density of 70.74 mW/m^2, substantiating electricity harvesting with simultaneous wastewater treatment. At similar influent COD, higher HRT is favourable for getting higher power density, whereas, there is an optimum range of HRT for getting maximum current density. Although power output obtained is still relatively low, the technology is improving rapidly and eventually could be useful to reduce the cost of small sewage and industrial wastewater treatment plant, which would be especially attractive in developing countries.

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