

redox shuttle mediated electron transfer (7), and electron transfer through conductive pili, also referred to as nanowires (9–11).

Cyclic voltammetry (CV) offers a rapid and proven method to discern whether bacteria use mobile redox shuttles to transfer their electrons, or pass the electrons "directly" through membrane associated compounds (6). For CV, a reference electrode is placed in the anode chamber of the MFC close to the anode (working electrode); the counter electrode (e.g., platinum wire) is preferably placed in the cathode chamber, but can also be placed in the anode chamber. A potentiostat is used to obtain a scan of potential. For bacterial suspensions, a scan rate of 25 mV s⁻¹ appears to be reasonable based on the work of several researchers (6, 73). For the analysis of mediators in biofilms, however, this scan rate needs to be decreased, possibly to 10 mV s⁻¹ and lower. This decrease can affect the accuracy of peak discrimination as the peaks tend to broaden.

The extent of the redox mediation and the midpoint potentials can be determined through analysis of (i) the MFC derived culture within its medium; (ii) the MFC culture after centrifugation and resuspension in physiological solution; and (iii) the supernatant of the centrifuged MFC culture. If a peak is found both in case (i) and (ii), it indicates a shuttle which is membrane associated. If a peak is found in case (i) and (iii), it indicates that a mobile, suspended shuttle is present. The size of the peaks, as integrated upon the voltammogram, either arbitrarily (as $\Sigma[\Delta I \Delta E \delta^{-1}]$) or through convolution analysis, does not correlate unequivocally to the extent of the membrane associated electron transfer and the mobile shuttle mediated electron transfer. This is caused by the restricted accessibility of the membrane associated shuttles for oxidation/reduction by the working electrode.

Presence of Nanowires. Electrically conductive bacterial appendages known as nanowires have only recently been discovered so their structure(s) are therefore not well studied or understood. Pili produced by some bacteria have so far been shown to be electrically conductive using scanning tunneling electron microscopy (11). There is no data at the present time whether nanowires can be detected or can be distinguished from adsorbed chemical shuttles via standard electrochemical methods such as CV. If electron shuttles associate with a nonconductive pili, or if the pili are covered with metal precipitates, they will be included in the CV measurements as membrane associated shuttles or may appear to be nanowires using STM. If redox shuttles are enclosed within the pilus' tubular structure they are unlikely to be detected using CV. Additional research will be needed to determine the best methods for detecting nanowires and determining their importance relative to other methods of electron transfer from cells to electrodes.

Outlook

MFC designs need improvements before a marketable product will be possible (26, 74). Both the issues identified above and the scale-up of the process remain critical issues. Most of the designs reviewed here cannot be scaled to the level needed for a large wastewater treatment plant which requires hundreds of cubic meters of reactor volume. Either the intrinsic conversion rate of MFCs will need to be increased, or the design will need to be simplified so that a cost-effective, large-scale system can be developed. Designs that can most easily be manufactured in stacks, to produce increased voltages, will be useful as the voltage for a single cell is low.

The success of specific MFC applications in wastewater treatment will depend on the concentration and biodegradability of the organic matter in the influent, the wastewater temperature, and the absence of toxic chemicals. Materials costs will be a large factor in the total reactor costs. Mainly

anodic materials commonly used in MFC reactors, such as graphite foams, reticulated vitreous carbon, graphite, and others, are quite expensive. Simplified electrodes, such as carbon fibers, may alleviate these electrode costs. The use of expensive catalysts for the cathode must also be avoided. Another crucial aspect is the removal of non-carbon based substrates from the waste streams: nitrogen, sulfur, and phosphorus containing compounds often cannot be discharged into the environment at influent concentrations. Similarly, even particulate organic compounds will need to be removed and converted to easily biodegradable compounds, as part of an effective wastewater treatment operation.

Applications. One of the first applications could be the development of pilot-scale reactors at industrial locations where a high quality and reliable influent is available. Food processing wastewaters and digester effluents are good candidates. To examine the potential for electricity generation at such a site, consider a food processing plant producing 7500 kg/d of waste organics in an effluent (14). This represents a potential for 950 kW of power, or 330 kW assuming 30% efficiency. At an attained power of 1 kW/m³, a reactor of 350 m³ is needed, which would roughly cost 2.6 M Euros (26), at current prices. The produced energy, calculated on the basis of 0.1 Euros per kWh, is worth about 0.3 M Euros per year, providing a ten-year payback without other considerations of energy losses or gains compared to other (aerobic) technologies. Moreover, decreased sludge production could substantially decrease the payback time.

In the long term more dilute substrates, such as domestic sewage, could be treated with MFCs, decreasing society's need to invest substantial amounts of energy in their treatment. A varied array of alternative applications could also emerge, ranging from biosensor development and sustained energy generation from the seafloor, to biobatteries operating on various biodegradable fuels.

While full-scale, highly effective MFCs are not yet within our grasp, the technology holds considerable promise, and major hurdles will undoubtedly be overcome by engineers and scientists. The growing pressure on our environment, and the call for renewable energy sources will further stimulate development of this technology, leading soon we hope to its successful implementation.

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