

# Continuous microbial fuel cells convert carbohydrates to electricity

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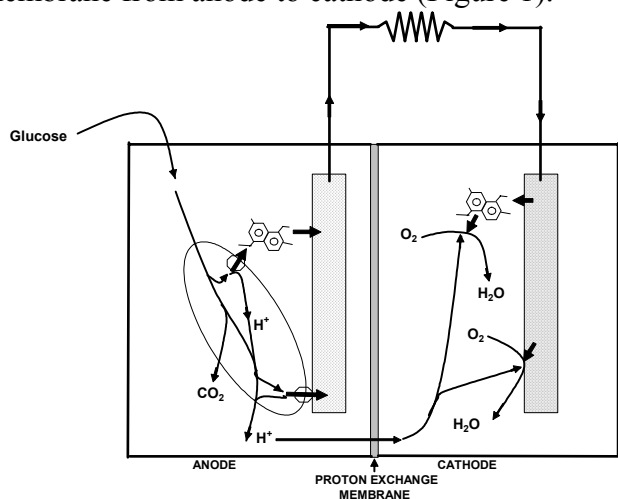
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**Abstract** Microbial fuel cells which are operated in continuous mode are more suitable for practical applications than fed batch ones. Three influent types containing carbohydrates were tested, i.e. a glucose medium, a plant extract and artificial wastewater. The anode reactor compartment yielding the best results was a packed bed reactor containing graphite granules. While in non-mediated batch systems power outputs up to  $479 \text{ W m}^{-3}$  of anode compartment could be attained; in continuous mode the power outputs were limited to  $49 \text{ W m}^{-3}$ . Cyclic voltammetry was performed to determine the potential of the in-situ synthesized bacterial redox mediators. Addition of mediators with a potential similar to the bacterial potential did not significantly alter the MFC power output, indicating a limited influence of soluble mediators for continuous microbial fuel cells. Maximum coulombic and energy conversion efficiencies were, for the continuous microbial fuel cell operating on plant extract at a loading rate of  $1 \text{ kg COD m}^{-3}$  of anode compartment per day, 50.3% and 26.0% respectively.

**Keywords** biofuel cell, glucose, sucrose, flow through, wastewater, plant sap

## Introduction

Bacteria gain metabolic energy by transferring electrons from an electron donor, such as glucose, to an electron acceptor, such as oxygen. The larger the potential difference between donor and acceptor, the larger the gain for the bacterium. In a microbial fuel cell (MFC), bacteria do not directly transfer their produced electrons to their characteristic terminal electron acceptor but these electrons are diverted towards an electrode (anode). The electrons are subsequently conducted over a resistance or power user towards a cathode and thus, bacterial energy is directly converted to electrical energy (Rao *et al.*, 1976). To close the cycle, protons migrate through a proton exchange membrane from anode to cathode (Figure 1).



**Figure 1** Working principle of a microbial fuel cell

Three main types are commonly distinguished: photo-autotrophic type biofuel cells (Tsujiura *et al.*, 2001), heterotrophic type biofuel cells, (Cooney *et al.*, 1996) and sediment biofuel cells (Bond *et al.*, 2002). Biofuel cells have characteristics similar to traditional power sources, as well as to anaerobic reactors. They can on the one hand be described by electrochemical parameters such as power density ( $\text{W m}^{-2}$  electrode surface or per  $\text{m}^3$  anode compartment), electrical current output and cell voltage and on the other hand by biological parameters such as the substrate loading rate ( $\text{kg m}^{-3} \text{ d}^{-1}$ ) (Rabaey *et al.*, 2003).

Direct electron transfer from bacteria to an electrode is hampered by overpotentials, which can be described as transfer resistances (Bard and Faulkner, 2001). In order to reduce these resistances, the specific surface of the electrodes needs to be increased, and/or redox mediators need addition to

the solution. A redox mediator is a compound that can be reversibly oxidized or reduced. Bacteria can use redox mediators to deposit their electrons onto a substrate they cannot directly reduce (Hernandez *et al.*, 2004). However, the addition of these mediators to the solution can represent a considerable cost, and their presence is usually not desirable for the effluent due to either colour or environmental effects.

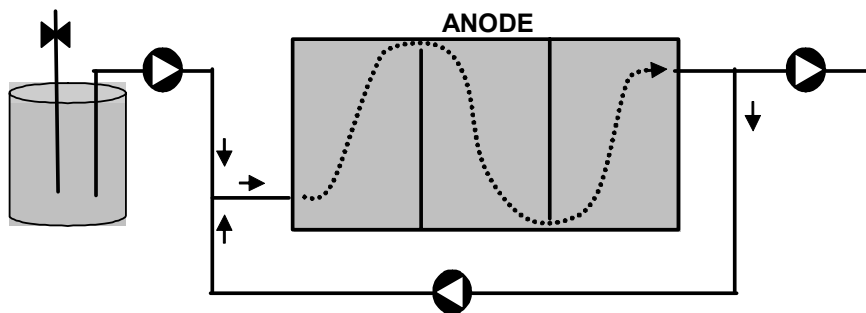
During a long term batch enrichment process for anodophilic consortia, the power output of a microbial fuel cell increased to  $4.31 \text{ W m}^{-2}$  anodic surface, corresponding to  $479 \text{ W m}^{-3}$  reactor (Rabaey *et al.*, 2004). Analysis of the bacterial community structure and identification of the bacteria suggested the evolution of the microbial community towards species that self-mediate this electron transfer. Analysis using cyclic voltammetry furthermore demonstrated that this self-mediating effect is dependent on two processes: (i) the expression of membrane-bound electron transfer components and (ii) the production of soluble redox mediators. An example of such a mediator is pyocyanin (Mavrodi *et al.*, 2001), produced by an isolated *Pseudomonas aeruginosa* species from the fuel cell. Using the two processes in parallel, bacterial consortia could transfer electrons towards an electrode with a maximum electron transfer efficiency up to 89%, calculated as coulombs gained as electricity per coulombs added as carbon source. This corresponds to an energetic efficiency up to 79%, calculated as joules gained as electricity per joules added as carbon source.

However, batch type microbial fuel cells are not suitable for applications such as wastewater treatment, since mediators and bacteria will flow out of the system. Therefore, the anode compartment needs to use an efficient biofilm on a large surface anode. Furthermore, the characteristics of the electrode need to be adapted to the biofilm and the used application. In this study, continuous flow microbial fuel cells were designed operating on several types of influents. After optimization of reactor parameters, the electrochemical characteristics of the bacteria were determined in order to define suitable mediators.

## Materials and methods

### Reactor setup

The microbial fuel cells were made of plexi glass elements that were bolted together as described previously (Rabaey *et al.*, 2003). The inner volume of the anode compartment was 0.250 l, the liquid volume varied between 0.080 and 0.220 l depending on the reactor configuration. Three anode types were used: (i) plain graphite anodes (8 x 4 x 0.6 cm) (Morgan, Belgium); (ii) granular anodes (3 mm average diameter) (Le Carbon, Belgium) without baffles (0.080 l of liquid volume); (iii) granular anodes, identical to previous set-up but with baffles (Figure 2) (0.080 l of liquid volume).



**Figure 2** Overview of the used set-up for continuous microbial fuel cells

The 250 ml cathode compartment contained a 100 mM phosphate buffered 50 mM potassium hexacyanoferrate solution (VWR, Belgium). Several types of cathode electrodes were used: (i) plain graphite cathodes (8 x 4 x 0.6 cm) (Morgan, Belgium); (ii) graphite felt electrodes (8 x 4 x 0.4 cm) (Alfa Aesar, Germany); (iii) granular cathodes (identical to anode granules), without baffles. The influent for the anode was provided at a loading rate of  $1 \text{ g l}^{-1} \text{ d}^{-1}$ , corresponding with a COD concentration between  $0.111 \text{ g l}^{-1}$  (0.080 ml anode liquid volume) and  $0.305 \text{ g l}^{-1}$  (0.220 ml anode liquid volume) for an influent volume of 0.720 l feed per day, corresponding to a hydraulic residence time between 2.7 and 7.3 h. A recirculation of  $8 \text{ l h}^{-1}$  was foreseen to provide sufficient anode turbidity. Measurements of the power output were performed using an Agilent HP 34970

data acquisition unit. Every 30 seconds, a full channel scan was performed and the data stored. External system resistance  $R$  was maximum  $100 \Omega$ , current was deducible as  $I = V \times R^{-1} = Q \times t^{-1}$  (Eq. 1) with  $I$  the current (A),  $V$  the voltage (V),  $Q$  the charge (C) and  $t$  time (s). Power output of the cells was calculated as  $P(W) = I \times V$  (Eq. 2). Energy production can then be expressed as  $\dot{E}(J) = P \times t$  (Eq. 3).

Two different inocula were initially tested: a sediment sample (Scheldt estuary, Belgium) grown in anaerobic nutrient broth prior to addition to the reactor, and a culture obtained from a microbial fuel cell on acetate, previously operated at LabMET.

### Influent preparation

Three influent types were prepared. The basic medium was M9 with a composition per litre: 6 g  $\text{Na}_2\text{HPO}_4$ ; 1 g  $\text{NH}_4\text{Cl}$ ; 0.5 g  $\text{NaCl}$ ; 0.2465 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ; 3 g  $\text{KH}_2\text{PO}_4$ ; 14.7 g  $\text{CaCl}_2$ . To this medium, a carbon source (glucose/maple syrup) was added to attain a loading rate of  $1 \text{ g COD l}^{-1}$  anode liquid volume per day. The artificial wastewater was prepared according to Jang *et al.* (Jang *et al.*, 2003), and contained per litre 0.56 g  $(\text{NH}_4)_2\text{SO}_4$ ; 0.2 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ; 0.42 g  $\text{NaHCO}_3$ ; 1 mg  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ; 20 mg  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ ; 14.7  $\text{CaCl}_2$ ; 6.8 g  $\text{KH}_2\text{PO}_4$ . This so-called artificial wastewater was supplied with glucose to obtain a reactor loading rate of  $1 \text{ g COD l}^{-1}$  anode liquid volume per day. To all influents, 1 ml of a trace element solution was added, containing per litre:  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  1g;  $\text{ZnCl}_2$  70 mg;  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  100 mg;  $\text{H}_3\text{BO}_3$  6 mg;  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$  130 mg;  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  2 mg;  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  24 mg;  $\text{Na}_2\text{Mo}_4 \cdot 2\text{H}_2\text{O}$  36 mg;  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  238 mg.

### Cyclic voltammetry

For the voltammetry analysis, 15 ml samples were taken from microbial fuel cells prior to the daily feeding, inserted in a test vial and flushed with nitrogen gas prior to the measurement. The cyclic voltammetry was performed as described previously (Park *et al.*, 2001). A potentiostat (Princeton Applied Research, USA, model 263a), branched to a PC was used (Princeton Applied Research, USA, SoftCorr III), at a scan rate of  $50 \text{ mV s}^{-1}$  in the potential range of  $-450$  to  $900 \text{ mV}$ . The working electrode was a  $5 \text{ cm}^2$  graphite rod, cleaned in ethanol and deionised water prior to use, the counter electrode was a platinum wire and an Ag/AgCl electrode (BAS, USA, MF-2052) was used as reference. All three were inserted in the test vial avoiding any contact between the electrodes. The mediators chosen for addition to the MFC were Resorufin (Fluka), Indigo carmine (Sigma), Safranin O (Sigma) and New Methylene Blue (UCB).

### Analysis

Samples were filtered through a syringe  $0.22 \mu\text{m}$  filter unit (Millex, USA). For analysis of the volatile fatty acids (VFA), an extraction in diethyl ether was performed (Greenberg *et al.*, 1992). The samples were analysed with a capillary FID (flame ionization detector) gas chromatograph, GC 8000 Carlo Erba Instruments (Wigan, UK), connected to a computer. The column used was an Alltech (Deerfield, USA) EC-1000 (30 m, I.D.:  $0.32 \text{ mm}$ ,  $d_f$ :  $0.25 \mu\text{m}$ ). The temperature was controlled at  $135 \text{ }^\circ\text{C}$  for the isotherm oven and  $200 \text{ }^\circ\text{C}$  for the detector and the injector. Nitrogen gas was used as the carrier gas at  $3 \text{ ml min}^{-1}$ . Samples were diluted 10 times and glucose assessed using ion chromatography (Dionex, CarboPac1 column with borate and amino trap, PAD ED40) (Groussac *et al.*, 2000). Gas chromatography (Intersmat IGC 120 MB) was used for determination of  $\text{CO}_2$  and  $\text{CH}_4$  in the headspace (Tsujimura *et al.*, 2001).  $\text{H}_2$  was measured using a Microtox exhaled hydrogen monitor (GMI, Germany), detection limit 5ppmv (Greenberg *et al.*, 1992). For the gas samples, 5 ml of anode headspace was obtained, after which the biofuel cell was flushed with nitrogen gas. pH electrodes (Metrohm, Switzerland) were installed to monitor the compartment pH. COD analysis was performed using the dichromate method (Greenberg *et al.*, 1992).

### Results

All loading rates and power output results are expressed as  $\text{W m}^{-3}$  of anode liquid volume to enable comparison between reactors that have a different content of electrode material per unit of reactor volume.

#### Influence of the initial inoculum

The reactors inoculated with the bacterial culture obtained from the sediment, demonstrated an average voltage of  $184 \pm 71 \text{ mV}$  versus  $133 \pm 21 \text{ mV}$  obtained with the former biofuel cell culture. Despite this non-significant difference between both inocula, all further tests were performed using

the sediment inoculum.

### Microbial fuel cells on glucose

A stable power output could be obtained within a short period of time for the reactors fed with glucose. Initially the power output was, on average,  $5.7 \text{ W m}^{-3}$ . After an interruption of the feeding for one day due to a technical problem, the power output increased gradually during a three week period to a daily average of  $37 \text{ W m}^{-3}$ . As verified by ion chromatography, glucose was always completely used. No accumulation of volatile fatty acids (VFA) was observed, the effluent concentrations gradually decreased to on average  $52 \pm 45 \text{ mg VFA l}^{-1}$ . Acetate was generally the only VFA present in significant quantities. The corresponding current generated represented up to  $49.0 \pm 0.3 \%$  of the added COD (Table 1). This represented an energetic efficiency of  $20 \%$  (Table 2). Declines were noted in the power output, which could be related to a visual deterioration of the influent. Indeed, when the latter became infected by acidifiers, the power output decreased. However, when influent was replaced a rapid (generally less than one hour time) return to the original voltage was noted.

**Table 1** Overview of COD conversion towards VFA and current in continuous MFCs operated at a loading rate of  $1 \text{ g COD l}^{-1}$  anode compartment per day. The calculations were made on the basis of average daily current and VFA output at the beginning and the end of the experimental period

| Reactor feeding |         | COD <sub>in</sub><br>(kg m <sup>-3</sup> d <sup>-1</sup> ) | COD <sub>out</sub> as current<br>(kg m <sup>-3</sup> d <sup>-1</sup> ) | COD <sub>out</sub> as VFA<br>(kg m <sup>-3</sup> d <sup>-1</sup> ) | COD <sub>out</sub> residual<br>(kg m <sup>-3</sup> d <sup>-1</sup> ) | COD <sub>out</sub> total<br>(kg m <sup>-3</sup> d <sup>-1</sup> ) | Recovery as current<br>(%) | Balance recovery<br>(%) |
|-----------------|---------|--|--|--|--|---|----------------------------|-------------------------|
| Glucose         | Initial | 1.000  | $0.165 \pm 0.036$  | $0.863 \pm 0.578$  | n.d.   | $1.028 \pm 0.579^*$   | $16.5 \pm 3.6$             | $103 \pm 58$            |
|                 | End     | 1.000  | $0.490 \pm 0.003$  | $0.096 \pm 0.057$  | $0.449 \pm 0.068$  | $0.939 \pm 0.068^*$   | $49.0 \pm 0.3$             | $94 \pm 7$              |
| Maple syrup     | Initial | 1.000  | $0.164 \pm 0.014$  | $0.684 \pm 0.104$  | n.d.   | $0.848 \pm 0.105^*$   | $16.4 \pm 1.4$             | $85 \pm 11$             |
|                 | End     | 1.000  | $0.541 \pm 0.013$  | $0.233 \pm 0.117$  | $0.306 \pm 0.086$  | $0.847 \pm 0.086^*$   | $54.1 \pm 1.3$             | $85 \pm 9$              |
| Wastewater      | Initial | 1.000  | $0.171 \pm 0.013$  | $0.827 \pm 0.302$  | n.d.   | $0.998 \pm 0.302^*$   | $17.1 \pm 1.3$             | $100 \pm 30$            |
|                 | End     | 1.000  | $0.391 \pm 0.009$  | $0.058 \pm 0.029$  | $0.679 \pm 0.090$  | $1.070 \pm 0.090^*$   | $39.1 \pm 0.9$             | $107 \pm 9$             |

COD calculated on the basis of current and VFA<sub>out</sub>-COD (\*) or total residual COD<sub>out</sub> (†)

n.d. not determined

### Microbial fuel cells on maple syrup

In parallel with the microbial fuel cells on glucose, other reactors were fed with M9 medium supplemented with maple syrup as carbon source. Initial power output was of the order of  $4 \text{ W m}^{-3}$ , corresponding with an energy production of  $0.09 \text{ kWh m}^{-3} \text{ d}^{-1}$ . This output gradually increased to  $49 \text{ W m}^{-3}$ , corresponding with an energy production of  $1.2 \text{ kWh m}^{-3} \text{ d}^{-1}$ . This implied a maximum cell potential of  $625 \text{ mV}$  and a current of  $6.3 \text{ mA}$  (Table 1 and 2). No accumulation of volatile fatty acids was observed, and concentrations decreased during the test period to  $17 \pm 11 \text{ mg VFA l}^{-1}$ . As in the glucose fed reactors, mainly acetate was present in the effluent

### Microbial fuel cells on artificial wastewater

A third series of microbial fuel cells was operated with artificial wastewater (Jang *et al.*, 2003), with glucose as carbon source. No large differences exist between this medium and the glucose medium used as described previously. However, the power output of these MFCs was lower than the power obtained for the other two substrates, namely  $24.7 \text{ W m}^{-3}$  after one month of operation (Table 1 and 2). As in the previous cases, no accumulation of VFA was observed, since effluent concentrations gradually decreased to about  $16 \pm 9 \text{ mg l}^{-1}$ . The VFA was mainly composed of acetate. There is clearly an influence of the basic medium onto the reactor performance.

### Influence of the electrode structure

To investigate the influence of the cathode structure onto the biofuel cell performance, two reactors were installed with either a graphite felt or plain graphite cathode. No significant differences in power output were noted between the graphite felt and the plain graphite cathode. During the first 50 hours of operation, the power output was, on average,  $8.8 \pm 0.4 \text{ W m}^{-3}$  and  $8.0 \pm 0.6 \text{ W m}^{-3}$  for the graphite felt and the plain graphite respectively. Maximum power output of the reactors was  $15.9 \text{ W m}^{-3}$  and  $15.2 \text{ W m}^{-3}$  for the graphite felt and the plain graphite respectively.

When graphite granules were used for the cathode, power output was initially of the same level

of the other cathodes tested, but the output rapidly decreased. A decolourization of the catholyte was observed, but this did not imply decreasing iron concentrations in solution. Presumably, the granular matrix did not allow sufficient proton transport to obtain sufficient water formation. Several different types of anode were used during the experimental period. Changing the anode from plate shaped to granular caused a two fold increase of the MFC voltage. The further addition of baffles to the anode compartment, in order to force a flow through the granular bed, allowed a further increase of the voltage to the values indicated previously.

**Table 2** Energy output of continuous MFCs on diverse substrate, without addition of redox mediators. The end period voltage indicated is the highest daily averaged voltage obtained.

| Reactor feeding |                | Energy <sub>in</sub><br>(kJ m <sup>-3</sup> d <sup>-1</sup> ) | Biofuel cell voltage<br>(mV) | Energy <sub>out</sub><br>(kJ m <sup>-3</sup> d <sup>-1</sup> ) | Energy recovery<br>(%) |
|-----------------|----------------|---|------------------------------|--|------------------------|
| Glucose         | Initial period | 15875   | 213                          | 490  | 3.1                    |
|                 | End period     | 15875   | 545                          | 3208   | 20.2                   |
| Maple syrup     | Initial period | 16200   | 172                          | 320  | 2.0                    |
|                 | End period     | 16200   | 625                          | 4220   | 26.0                   |
| Wastewater      | Initial period | 15875   | 201                          | 436  | 2.7                    |
|                 | End period     | 15875   | 445                          | 2139   | 13.5                   |

### Cyclic voltammetry for mediator selection

Cyclic voltammetry was performed onto the bacterial culture derived from the glucose fed microbial fuel cells. Oxidation and reduction peaks were found, indicating electrochemical activity at a potential of approximately -50 mV versus standard hydrogen electrode. Redox mediators, having a standard redox potential near the measured activity potential of the bacteria, were applied at a concentration of 50 µM. Upon addition of the mediator to the anode, a fast decolourisation was noted, indicating reduction of the mediator. No significant effects of the mediators onto the MFC power output were noted in any of the cases. These results differ from the previous findings obtained for batch systems (Choi *et al.*, 2003; Park and Zeikus, 1999; Roller *et al.*, 1984).

## Discussion

### Removal of COD by microbial fuel cells

Up to 50 % of the COD present in the influent was removed as electricity (Table 1). The remainder of the COD was either present in the effluent as acetate, or not detected. The COD which is lacking is most likely hydrogen gas, which was not measured using these continuous reactors. The fact that the acetate present in the effluent is reasonably low, demonstrates the capability of the microbial fuel cell to biodegrade substrates such as glucose further down than simple fermentation to acetate and other VFA. This opens possibilities for microbial fuel cells to be used for COD removal.

A discrepancy exists between the coulombic efficiencies obtained (Table 1) and the energetic efficiencies obtained (Table 2). Generally, for wastewater treatment, COD removal is the prime parameter of importance. This COD removal can be expressed as coulombic efficiency. However, the added value of the produced power can be as important in terms of economic feasibility of the WWTP. To obtain a high energetic efficiency, both voltage and current need to be of sufficient magnitude. This implies that the resistance over the microbial fuel cell cannot be too high, neither too low. Thus far, almost all studies expressed microbial fuel cell output by mA m<sup>-2</sup> or W m<sup>-2</sup> of anode. In order to compare this technology with other existing technologies, the calculations should be remade based on W m<sup>-3</sup> of anode liquid volume. This calculation should clarify whether the loading rates used and the power outputs observed have significance for practice. Furthermore, this would enable a comparison of the different studies performed.

Studies on microbial fuel cells have thus far always applied large resistances of over 500 Ω, with the exception of two publications by our group (Rabaey *et al.*, 2003, 2004). This generated large cell potentials, but low currents. Hence, total power output of the microbial fuel cells was low. Further research is needed to determine the optimal resistance over the MFC generating the largest power. This optimal resistance will more than likely be depending on the application, the inoculum, the type of reactor, the substrate and the potential losses within the microbial fuel cell.

### Applicability of the used substrates

The substrates used were chosen as model components towards practical applications. The data obtained indicates energy recoveries up to 26% at the moderate loading rate of 1 kg m<sup>-3</sup> d<sup>-1</sup>.

Microbial fuel cells for wastewater treatment, based on this study, would carry several advantages: (i) COD is removed (ii) useful power can be obtained (iii) no aeration is needed (iv) no complicated equipment for processing of biogases is needed. These advantages compete with the disadvantage of a larger process complexity of the reactor in comparison to traditional wastewater treatment.

### Importance of redox mediators

While in batch systems, the improvement of the power output through the addition of redox mediators was repeatedly reported (Park and Zeikus, 1999; Roller *et al.*, 1984), for the continuous microbial fuel cells no significant effect was observed. Clearly, the addition of soluble mediators to the anode is not a desirable strategy for continuous MFCs. Hence, the possibility of immobilizing the mediator onto either active biomass or onto/into electrode material should be further elaborated.

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