Generation of electricity from the hydrogen produced by fermentative utilization of different substrates using *Enterobacter aerogenes* NCIM 2340

presented by

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1. Introduction

- The limited fossil fuel- the use of hydrogen is an attractive alternate source.
- Advantages- environmentally clean, renew ability, liberates large amount of energy and easily converted to electricity by fuel cells, only waste product being water.
- The existing commercialized physicochemical hydrogen production processes are not economic.
- Biological hydrogen production processes are found to be more environment friendly and less energy intensive.
- Microbial production of H_2 using variety of substrates has gained substantial interest in recent years (Nath and Das, 2004).
- Microbial H_2 production has the dual benefits- generating a clean fuel and reducing waste.

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- Interconversion between these two forms of energy suggests on-site utilization of hydrogen to generate electricity (Liu et al., 2005, Angenent et al., 2004).
- The photofermentation method has been used since a long time.
- Higher yields can be achieved using this and by using pure enzymes, but neither of these methods so far show promise for economical production of hydrogen (Miyake et al., 1999, Woodward et al., 2000).
- Compared to this the dark fermentation involves the fermentation using various microbes.
- A limited number of microorganisms have been identified which are capable of producing H_2.

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- But as far as the suitability towards commercialization is considered a facultative organism are desired one.
- The microbial fuel cell (MFC) uses hydrogen and oxygen to produce electricity (Bond et al., 2002, Bond and Lovely, 2003).
- The hydrogen production ability of microbes can be utilized in MFCs- new path to electricity production.
- The pure cultures like *Clostridium cellulolyticum, C. thermocellum* (Niessen et al., 2005), *E. coli* (Schroder et al., 2003), *C. butyricum* and *C. beijerinckii* (Niessen et al., 2004) have been tried by various scientists.
- Currently, MFC research is strongly growing w. r. t. substrates, microbial cultures, electrodes and ion exchange membranes (Rosenbaum 2006, Niessen et al., 2006, Schroder, 2007, Rosenbaum et al., 2007).

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- The direct oxidation of hydrogen has potential advantages- does not require the separation and purification of the gas.
- Carbohydrates, domestic wastewaters, proteins have been tried as substrates in MFCs due to environmental concerns (Niessen et al., 2004, Logan, 2004, Heilmann and Logan, 2006).
- Apart from pure strains, anaerobic sludge, marine sediments, soils produces high current (Park and Zeikus, 2002, Niessen et al., 2006).
- The production and continuous supply of hydrogen in fuel cells is possible using fermentative microorganisms. MFCs show promise, as a method to treat waste water as well as to produce electricity (Liu et al., 2004, Jang et al., 2004).

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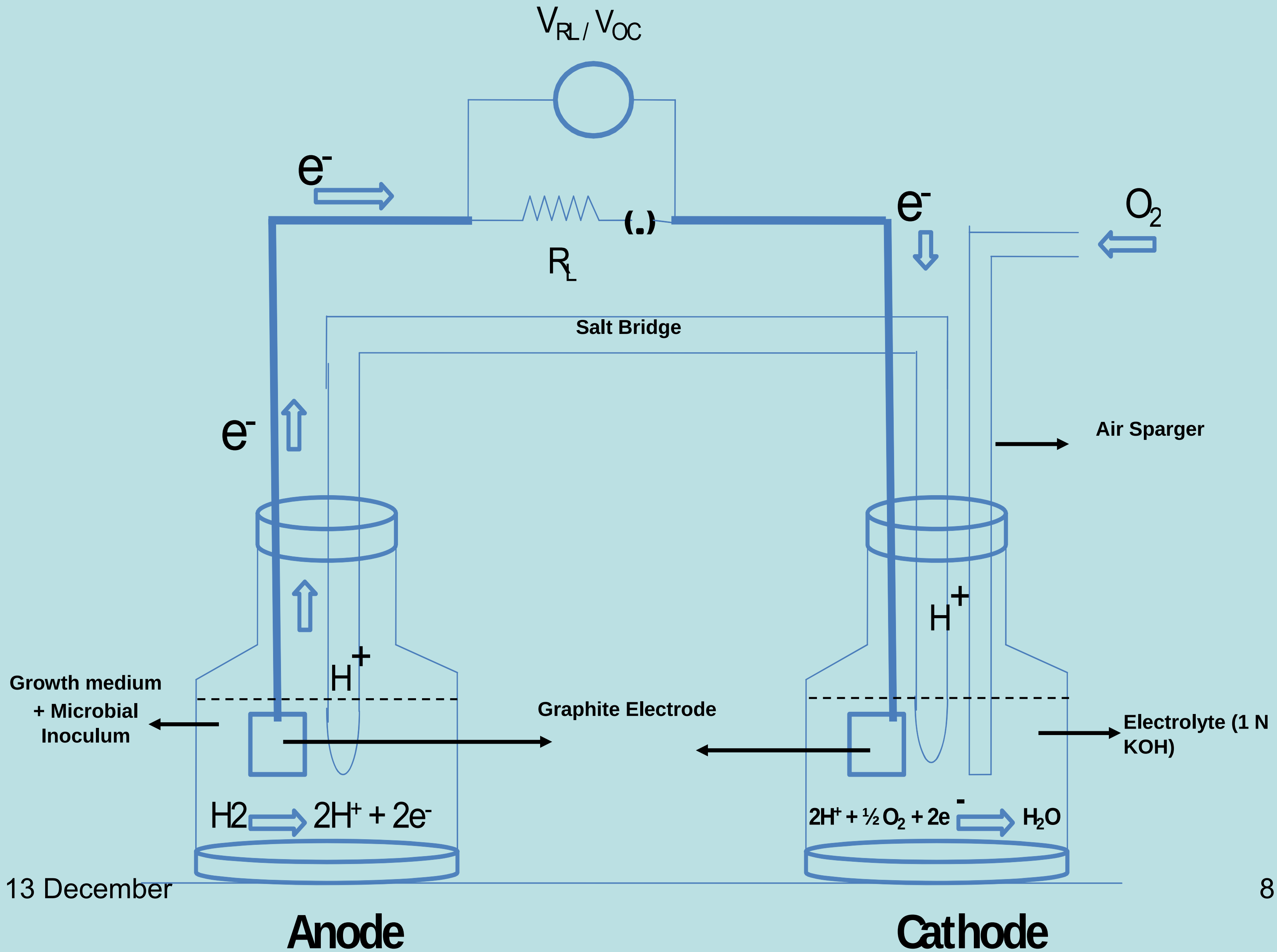
- Microorganisms are found to be able to produce their own electron mediators- enhances electron transfer in MFCs (Gil et al., 2003).
- Our study dealt with design of mediatorless microbial fuel cell and linking hydrogen production from a industrial byproducts to electricity generation using a MFC.
- The pure cultures are supposed to have high reproducibility and a comparably low degree of complexity. Also, towards commercialization a facultative organism are considered to be the desired one.
- Thus, we tested the feasibility of *Enterobacter aerogenes* NCIM 2340 for electricity production using different using dairy whey and molasses under mesophilic conditions.

2. Experimental

2.1 Design and set up of MFC

- Two glass bottles (500 ml capacity each)
- U-shaped salt bridge
- Graphite electrodes (length = 12.0 cm, diameter = 1.2 cm)
- Copper wire was inserted in electrodes to connect the circuit.
- Voltmeter was connected to measure the voltage [Fig. 1]
- Anode chamber- 300.0 ml of growth medium + 10 % inoculum + stirring
- Cathode chamber- equal volume of 1.0 N KOH as an electrolyte + air
- The MFC was operated at $30^{o}C$ for 72 h.

Figure 1: Design and set up of Microbial Fuel Cell (MFC)



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2.2 Microbial inoculum, growth medium and culture conditions

- Inoculum - *Enterobacter aerogenes* NCIM 2340
- Substartes- dairy whey and molasses. These were sampled from Shri Warana Dugdh Sahakari Sangh (Dairy), Amrutnagar and Shri Warana Sugar Factory, Warananagar.
- The concentration of sugar in molasses was 40 %. It was diluted by 10 times to make final concentration to 4.0 %.
- Synthetic medium- glucose + mineral solution

The composition [per 1.0 L of d/w]: Glucose 10.0 g, Na acetate 1.4 g, FeCl3 12.0 g, NH4Cl 0.30 g, NaH2PO4.H2O 0.66 g, KCl 0.120 g, NaHCO3 2.0 g [pH 7.0].

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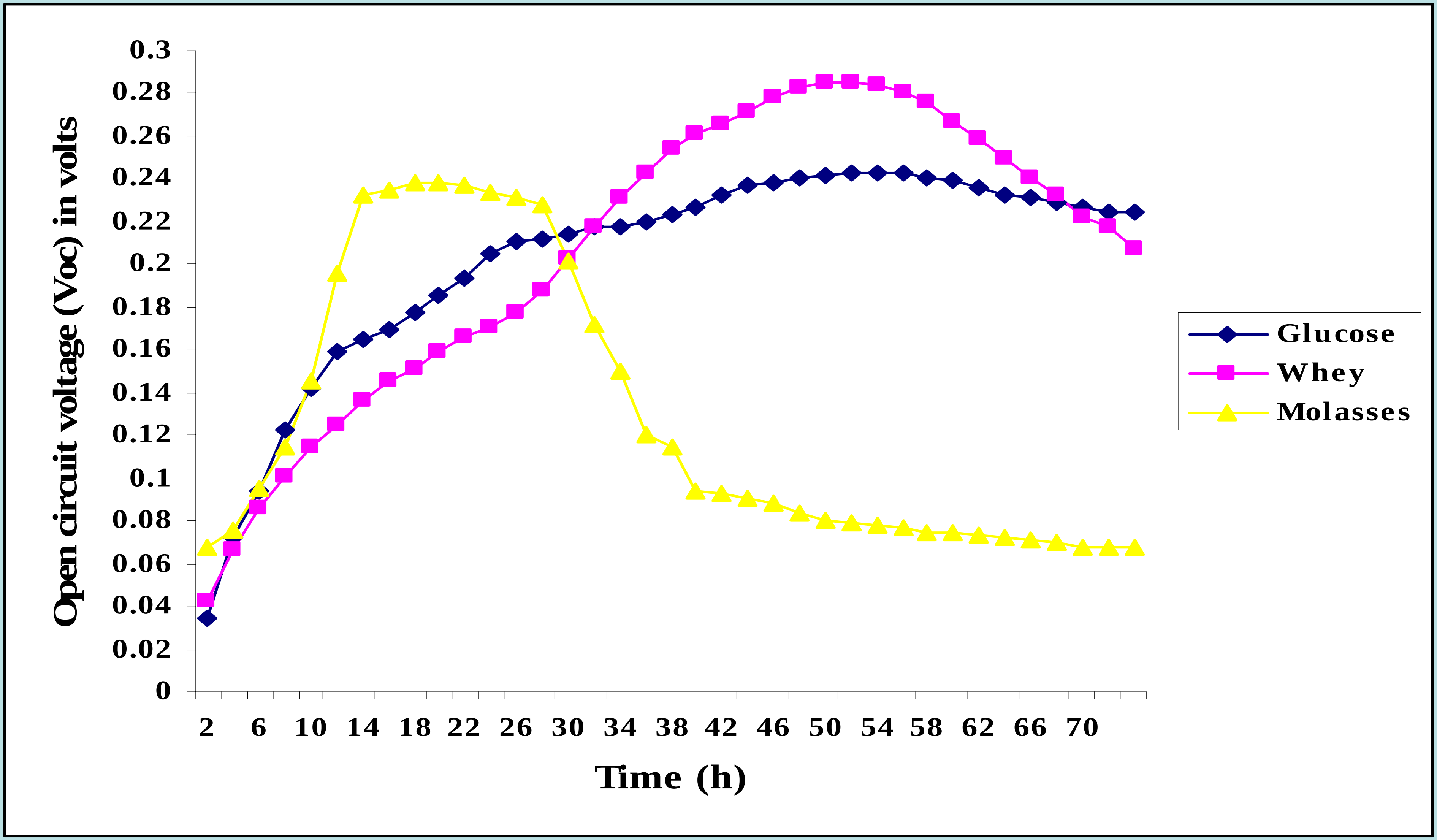
2.3 Parameters measured for studying performance of MFC

- The voltage generated with these substrates was monitored for 72 h using a Voltmeter connected to MFC.
- The open circuit voltage (Voc) was measured at regular time intervals.
- The circuit was completed with a fixed load resistance of 100 Ω and V_{RL} was measured.
- At the maximum performance of MFC, the current (I) was calculated at 100 Ω (RL) from the voltage (V_{RL}).
- The power output was calculated with a fixed load resistance of 100 Ω. Power density, P (W), was obtained according to P = IV.

3. Results and Discussion

- The microbial utilization of nutrients results in generation of electrons into medium and gives rise to the flow of electrons into the external circuit.
- The variation of open circuit voltage (Voc) in MFC with these substrates was measured for 72 h (Figure 2).
- Initial circuit voltage in the range of 0.02-0.07 V was immediately generated (Figure 2).
- The initial voltage might be due to chemical and biological factors based on the difference of the potential between the two chambers.
- Thereafter, the voltage rapidly increased due to biological activity, and stabilized at different values for different substrates and then there was gradual decrease in the voltage with respect to time. This behavior of Voc with time 't' is due to the utilization of nutrients from medium by microbes.

Figure 2: Variation in Open circuit voltage (Voc) with different substrates



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- The maximum voltage at 100 Ω resistor (VRL) with glucose, whey and molasses was found to be 0.060 V, 0.074 V and 0.055 V respectively.
- The current generation is based on in situ oxidation of microbially synthesized hydrogen at graphite electrodes.
- Maximum current- 0.60 mA, 0.74 mA and 0.55 mA and power outputs- 36 mW, 54 mW and 30 mW were obtained with glucose, dairy whey and molasses respectively under batch experiments (Table 1).
- The maximum power output with different substrates varies for the two-chambered MFC, indicating that the type of substrate can affect power output in MFC.
- These results demonstrate that the substrate composition affected microbial kinetics and therefore the maximum power output.

Table 1: Current and power output (at R=100Ω) in MFC with different substrates inoculated with Enterobacter aerogenes NCIM 2340

Substrate	**Current (mA)**	**Power output (mW)**
Glucose	0.60	36
Dairy whey	0.74	54
Molasses	0.55	30

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- So far, electron transfer without mediators has always been described as not feasible. With electron mediators the power output is found to be increased significantly (Park and Zeikus, 2002).
- In our study the voltage obtained was consistent for more than three days with a mediatorless fuel cell.
- The current and power output obtained is comparable with pure cultures that have been used by various scientists (Schroder et al., 2003, Niessen et al., 2004, Kim et al., 2000, Choi et al., 2003, Kim et al., 2002, Bond and Lovley, 2003, Chaudhuri and Lovley, 2003, Rabaey et al., 2005).
- The low power output was directly attributed to the higher internal resistance of the salt bridge system.

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- A power density of the proton exchange membrane MFC is up to two orders of magnitude larger than salt bridge MFC if operated under similar experimental conditions (Min et al., 2005).
- This indicates that optimizing power generation in MFCs will require maximizing proton transport rates by reducing the internal resistance of the system.
- Use of membranes will definitely increase the power output under similar conditions.
- The results suggest that it is feasible to link biological hydrogen production with electricity production using MFCs in order to achieve both wastewater treatment and bioenergy production.

4. Conclusion

- The *Enterobacter aerogenes* can be used as a microbial source for electricity generation in microbial fuel cells using different organic substrates. The advantages- flexible substrate utilization and simplicity in handling.
- The large scale engineering of such technologies would provide methods of generating electricity or hydrogen from wastewaters as well as it may help to reduce the costs of wastewater treatment in the developing nations.
- Further research and development is in progress for maximizing hydrogen production using batch and continuous flow reactors; construction and testing of a novel MFC for electricity production.
- High current output and flexible fuel utilization are prerequisites for a successful implementation of microbial fuel cells in biomass and waste utilization. With the here presented work we hope to be able to get one step closer to this aim.

5. References

- Angenent L.T., Karim K., Al-Dahhan M. H., Wrenn B. A. and R. Domiguez-Espinosa, 2004, Production of bioenergy and biochemicals from industrial and agricultural wastewater, *Trends in Biotechnology,* 22(9), 477-485.
- Bond D.R., Holmes D.E., Tender L.M. and D. R. Lovley, 2002, Electrode reducing microorganisms that harvest energy from marine sediments, *Science,* 295, 483-485.
- Bond D. R. and D. R. Lovely, 2003, Electricity production by *Geobacter sulfurreducens* attached to electrodes, *Applied and Environmental Microbiology,* 69, 1548-1555.
- Chaudhuri, S.K. and D.R. Lovley, 2003, Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells, *Nature Biotechnology*, 21, 1229-1232.
- Choi Y., Kim N., Kim S., and S. Jung, 2003, Dynamic behaviors of redox mediators within the hydrophobic layers as an important factor for effective microbial fuel cell operation, *Bulletin of Korean Chemical Society,* 24, 437-440.

cont…..

- Gil G.C., Chang I.S., Kim B.H., Kim M., Jang J.K., Park H.S. and H.J. Kim, 2003, Operational parameters affecting the performance of a mediator-less microbial fuel cell, *Biosensors and Bioelectronics,* 18, 327-334.
- Hallenbeck P. and J. R. Benemann, 2002, Biological hydrogen production: fundamentals and limiting processes, *International Journal of Hydrogen Energy,* 27, 1185-94.
- Heilmann J. and B. E. Logan, 2006, Production of Electricity from Proteins Using a Microbial Fuel Cell, *Water Environment Research,* 78(5), 531-537.
- Jang J.K. Chang I. S., Jang J. K., Kim B. H., Kang K. H., Pham T. H., Moon H. S. and K. S. Cho, 2004, Construction and operation of a novel mediator- and membrane-less microbial fuel cell, *Process Biochemistry,* 39, 1007-1012.
- Kim N., Choi Y., Jung S. and S. Kim, 2000, Development of Microbial fuel cells using *Proteus vulgaris, Bulletin of Korean Chemical Society,* 21(1), 44-48.
- Kim, H.J., Park H.S., Hyun M.S., Chang I.S., Kim M. and B.H. Kim, 2002, A mediator-less microbial fuel cell using a metal reducing bacterium, Shewanella putrefaciens, *Enzyme and Microbial Technology,* 30, 145-152.

cont…..

- Logan B.E., 2004, Feature article: biologically extracting energy from wastewater: biohydrogen production and microbial fuel cells, *Environmental Science Technology,* 38, 160-167.
- Liu H., Ramnarayanan R. and B.E. Logan, 2004, Production of electricity during wastewater treatment using a single chamber microbial fuel cell, *Environmental Science Technology,* 38, 2281-2285.
- Liu H. and B.E. Logan, 2004, Electricity generation using an aircathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane *Environmental Science Technology,* 38 (14), 4040-4046.
- Liu H., Grot S. and B. E. Logan, 2005, Electrochemically Assisted Microbial Production of Hydrogen from Acetate, *Environmental Science Technology,* 39, 4317-4320
- Miyake J., Masato M. and A. Yasuo, 1999, Biotechnological hydrogen production: research for efficient light energy conversion, *Journal of Biotechnology,* 70, 89-101.
- Min B. and B.E. Logan, 2004, Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell, *Environmental Science Technology,* 38 (21), 5809-5814.

cont…..

- Min B., Cheng S. and B. E. Logan, 2005, Electricity generation using membrane and salt bridge microbial fuel cells, *Water Research,* 39, 1675-1686.
- Nandi R. and S. Sengupta, 1998, Microbial production of hydrogen: an overview, *Critical Reviews in Microbiology,* 24, 61-84.
- Nath K. and D. Das, 2004, Improvement of fermentative hydrogen production: Various Approaches, *Applied Microbiology and Biotechnology,* 65, 520-529.
- Niessen J., Schroder U. and F. Scholz, 2004, Exploiting complex carbohydrates for microbial electricity generation - a bacterial fuel cell operating on starch, *Electrochemistry Communications,* 6, 955-958.
- Niessen J., Schroder U., Harnisch F. and F. Scholz, 2005, Gaining electricity from in situ oxidation of hydrogen produced by fermentative cellulose degradation, *Letters in Applied Microbiology,* 41, 286-290.

cont…..

- Niessen J., Harnisch F., Rosenbaum M., Schroder U. and F. Scholz, 2006, Heat treated soil as convenient and versatile source of bacterial communities for microbial electricity generation, *Electrochemistry Communications,* 8, 869-873.
- Oh S.E., Min B. and B.E. Logan, 2004, Cathode performance as a factor in electricity generation in microbial fuel cells, *Environmental Science Technology,* 38 (18), 4900-4904.
- Park D.H. and J.G. Zeikus, 2002, Improved Fuel Cell and Electrode Designs for Producing Electricity from Microbial Degradation, *Biotechnology Bioengineering,* 81, 348-355.
- Park, D. H. and J. G. Zeikus, 2002, Impact of electrode composition on electricity generation in a single-compartment fuel cell using *Shewanella putrefaciens, Applied Microbiology and Biotechnology,* 59, 58-61.
- Rabaey K., Boon N., Hofte M. and W. Verstraete, 2005, Microbial Phenazine Production Enhances Electron Transfer in Biofuel Cells, *Environmental Science Technology* 39, 3401-3408

cont…..

- Rosenbaum M., Zhao F., Schroder U.,and F. Scholz, 2006, Interfacing Electrocatalysis and Biocatalysis with Tungsten Carbide: A High-Performance, Noble- Metal-Free Microbial Fuel Cell, *Angewandte Chemie,* 118, 1-4.
- Rosenbaum M., Zhao F., Quaas M., Wulff H., Schroder U. and F. Scholz, 2007, Evaluation of catalytic properties of tungsten carbide for the anode of microbial fuel cells, *Applied Catalysis B: Environmental,* 74, 262-270.
- Schroder U., Niessen J. and F. Scholz, 2003, A Generation of Microbial Fuel Cells with current outputs boosted by more than on order of magnitude, *Angewandte Chemie,*115, 2986-2989.
- Schroder U., 2007, Anodic electron transfer mechanisms in microbial fuel cells and their energy efficiency, *Physical Chemistry Chemical Physics,* 9, 2619-2629.
- Woodward J., Orr M., Cordray K. and E. Greenbaum, 2000, Enzymatic production of biohydrogen, *Nature,* 405, 1014-1015.

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