1. Introduction

Microbial fuel cells are devices that convert chemical energy into electrical energy, using bacteria as a catalyst to oxidize organic and inorganic matter without the inefficiencies that arise from combusting fuel to produce electricity (Tiehm *et al*., 2001; Hong*et al*., 2008). MFCs provide new opportunities for the energy production from reduced biodegradable compounds. They are unique subset of fuel cells that take advantage of microbial metabolism to generate fuels for commercial fuel cells or electricity directly.

An MFC is a bio-electrochemical system which produces the current from oxidation of biodegradable organic compounds using microorganisms called electrogens that are capable to transfer electrons outside their cell. *Geobater sulfurreducens* is a best choice to evaluate the adaptation for enhanced power production is feasible as *G.sulfurreducens* are enriched on anodes from the complex communities of microbes when there is a high selective pressure for high rates of current production at higher columbic efficiencies. (Gregory *et al*., 2005; Bond et al., 2002; Holmes *et al*., 2004; Jung and Regan. 2007; Lee *et al*.,2008; Liu *et al*.,2007; Tender *et al*.,2002).

1.1. History of MFC:-

- M. Potter (1911) - Convienced the idea of using microbial cells in an attempt to produce electricity. He had idea of trying to harvest this newfound source of energy for human use.
- Barnet Cohen (1931)- Drew more attention to the area when number of microbial half fuel cells when connected in series they were capable of producing over 35 volts, though with a 2 milliamps current.
- DelDuca *et al*. used hydrogen that is produced by the glucose fermentation by *Clostridium butyricum* as the reactant at the anode of hydrogen and air fuel cell.
- Suzuki *et al*. (1976) - The current design concept of an MFC came in existence.
- MJ Allen and H. Peter Bennetto (late 1970s) - The idea of Suzuki was picked up and studied in detail later.
- H. Peter Benneto (1980s) – build an understanding of how fuel cells operate.
- B-H. Kim (1900s) – Discovered that certain species of bacteria were electrochemically active and didn't require the use of mediator molecule to transport electrons to the electrodes.
- By 1999, researchers in South Korea discovered MFC milestone.
- In May 2007, the Universityof Queensland, Australia completed its prototype MFC as a cooperative effort with Foster's Brewing.
- Currently, researchers are working to optimize electrode materials, combinations and types of bacteria and transfer of electron in microbial fuel cell.
- Almost around 100 years the idea of energy production by harnessing the bacteria.

1.2 Microbial Fuel Cell Principle :-

The main components of microbial fuel cells are:

1. Anode
2. Electrolyte
3. Cathode

A typical MFC consists of two compartments

a) Anodic half cells
b) Cathodic half cells

MFC is dissimilatory metal reduction. (Bretschger *et al*., 2007; Bond *et al.*, 2002; Chaudhuri and Lovley, 2003). They are separated by a salt bridge, selectively permeable or specific membrane. The anodic chamber consists of the microbes that are suspended under anaerobic conditions in the anolyte and the cathodic chamber consists of electron acceptor like oxygen (Rittmann, 2008). From the process of oxidation the electrons released are conveyed to the anode. Electron transfer to the anode can be accomplished by indirect transfer using shuttling agents or electron mediators (Lovley, 2008; Logan *et al*., 2006. Rittmann, 2008 ;), through direct transfer by bacterial structures called, nanowires or directly by the cell. These electrons are directed to the cathode across an electrical circuit and for every electron conducted,a proton is transported across the cathode membrane for completion of the reaction and sustaining the electric current.

1.3. Design of Microbial Fuel Cell:-

Variety ofscalable designs for constructing an MFC has proposed by researchers. The traditional, dual chambered (H- shaped) MFC is commonly adopted configuration, in which two chambers are connected by means of a tube containing a separator membrane (Logan *et al*., 2006). Initially reactors used a salt bridge) as ion exchange channel between the cathode and anode chamber and these were replaced by cation/ proton exchange membrane. The best known designs include an upflow tubular type MFC (Rabaey *et al*., 2005), a flat plate design (Min and Logan, 2004), a stacked MFC (Aelterman*et al*., 2006), and a U-tube MFC (Zuo *et al*., 2008).

A sediment-type MFC, that uses sediments and overlying water as anode and cathode respectively, has recorded power densities as 55mW/m² with sea water (Scott *et al*., 2008).

Fig 1: Patented two-chamber scalable MFC designs from A) Chiao, *et al*., [19], B) Ringeisen, *et al*., [20].

2...MFC Microbiology:-

In the new emerging field of microbial ecology electrochemically active microbes is still in its infancy. These are based on anodophilic bacteria and possible interspecies electron transfer. Such bacteria are referred to as exoelectrogens.

Exoelectrogens are those microorganisms that have the ability to transfer electrons exocellularly. Utilization of such exoelectrogens is being currently researched in the development of MFCs which has potential to convert organic material such as activated sludge from waste water treatment into ethanol, electric current and hydrogen gas etc.

Transfer of electrons exocellularly are in three ways:

1. Self- produced mediators
2. Membrane bound electron carrier
3. Nanowires (connective appendages)

Understanding of electron transfer by bacteria to electrodes came from studies of metal reducing bacteria like *Geobacter* and *Shewanella* species can produce electricity in MFCs. Biochemical and genetic characterizations indicate that outer-membrane cytochromes can be involved in exogenous electron transfer. There are some bacteria that produce and use soluble electron shuttles that eliminate the need for direct contact between the cell and electron acceptor.

For ex: -Phenazine production by a strain of *Pseudomonas aeruginosa* stimulates electron transfer for several bacterial strains.

Nanowires introduce a new dimension to study extracellular electron transfer. These are conductive, pilus- like structures, and are identified in *Geobacter sulfurreducens* PCA, *Shewanella oneidensis* MR-1,, a phototrophic *Cyanobacterium synechocystis* PCC6803,and the thermophilic fermenter *Pelotomaculum thermopropionicum* appeared to be involved directly in extracellular electron transfer.

Those bacteria thrive in MFC biofilms, either through electron transfer to anode, or by non-electrochemical metabolisms such as fermentation or symbiotic relationships with other bacteria, are distributed across the phylogenetic subclasses. In this anode is used instead of the normal terminal electron acceptors.

3.Electricigens:-

Electricigens are the micro-organisms that are able to oxidize organic matter to CO_2 at the same time that electrons are transferred to electrodes. Electricigens are able to convert renewable biomass and organic detritus into electricity without consume the fuel wasting energy in heat form. The ability of *Geobacter* species is to oxidize organic compounds completely with an electrode that serve as the electron acceptor, and to conserve energy to support growth this metabolism, represents a novel form of microbial respiration. The capacity to conserve energy from this metabolism for the sustainability of long- term of microbial fuel cell is essential. Hence microorganisms that conserve energy from electron transfer to a referred to as electicigens.

Several electricigens outside the *Geobacteraceae* are described such as:

1. *Pseudomonas*
2. *Shewanella putrefaciens*
3. *Rhodoferax ferrireducens*
4. *Desulfobulbus propionicus*

R.ferrireducens was isolated from subsurface sediments as a Fe^{3+} reducer[67], oxidizes sugars such as glucose, fructose, lactose and xylose to CO^2 with THE RECOVERY OF 80% of electrons derived from sugar oxidation as electricity.

Desulfobulbus propionicus electricigen is discovered from molecular analysis of the anode surfaces of sediment microbial fuel cell. Electrodes harvesting electricity from sediments with high concentrations of sulphide (S^{2-}) were colonized by microorganisms in the family *Desulfobulbaceae.*

3.1 Other electicigens

The *Geobacter* species has the ability to completely oxidize organic compounds with an electrode that serve as acceptor of electron and conserve energy to support growth from metabolism and a novel form of respiration of microbe is represented. The electron donors are oxidized such as acetate which is the key constituents of carbon and electron flow during organic matter anaerobic degradation makes it possible to effectively convert complex organic matter to electricity with *Geobacter* species combination and appropriate fermentative microorganisms oxidize organic compounds fermentative microorganisms. For the long- term sustainability of a microbial fuel cell it is essential to be capable to conserve energy from this metabolism...

The energy is conserved by microorganisms to support growth from methane production are known as methanogens. Hence it is suggested that microorganisms that conserve energy of electron transfer to the electrode is known as electricigens. Microorganisms associated with anodes are termed as electrodophile and anodophile. The microorganisms that do not conserve energy for growth support from the transfer of to electrodes are also associated with these terms. The anodophile is not specific enough to describe electricigens respiration because many of the microorganisms might attach to the surface of anode but it's not necessary to contribute to the production of electricity Electricigens other than *Geobacteraceae* are *Rhodoferax ferrireducens* was isolated from the subsurface sediments as an Fe^{3+} reducer, oxidized sugars such as glucose, fructose, sucrose,, lactose and xylose to carbon dioxide with over 80% recovery of electrons that are derived from sugar oxidation as electricity. This organism is of special interest because of attempts to convert sugars to electricity in microbial fuel cell. When the electrical connection in the *R.ferrireducens* fuel cell is disconnected for 36 hours, there is no means of energy generation or power production leaving of *R. ferrireducens*. *Rhodoferax* is an ideal candidate for pure culture system for converting sugars to electricity.

Another electricigen is *Desulfobulbus propionicus* and is discovered from molecular analysis of the anode surfaces of sediment microbial fuel cell. *D. propionicus* is a pure culture representative of *Desulfobulbaceae* family. It is revealed that oxidized S^o to sulphate ($SO4^{2-}$) with an electrode serving as the sole acceptor. It is an important reaction in sediments at the anode surface with concentrations of sulphide. This is because produced sulphide might be abiotically reacting with electrodes producing S^o. This abiotic reaction harvests two of the eight electrons that are potentially available from sulphide oxidation. Oxidation of S^o to sulphate extracts six electrons and sulphate is regenerated as an electron acceptor for the further microbial reduction. (Derek R. Lovley Volume 1, Number 7, 2006 / Microbe)

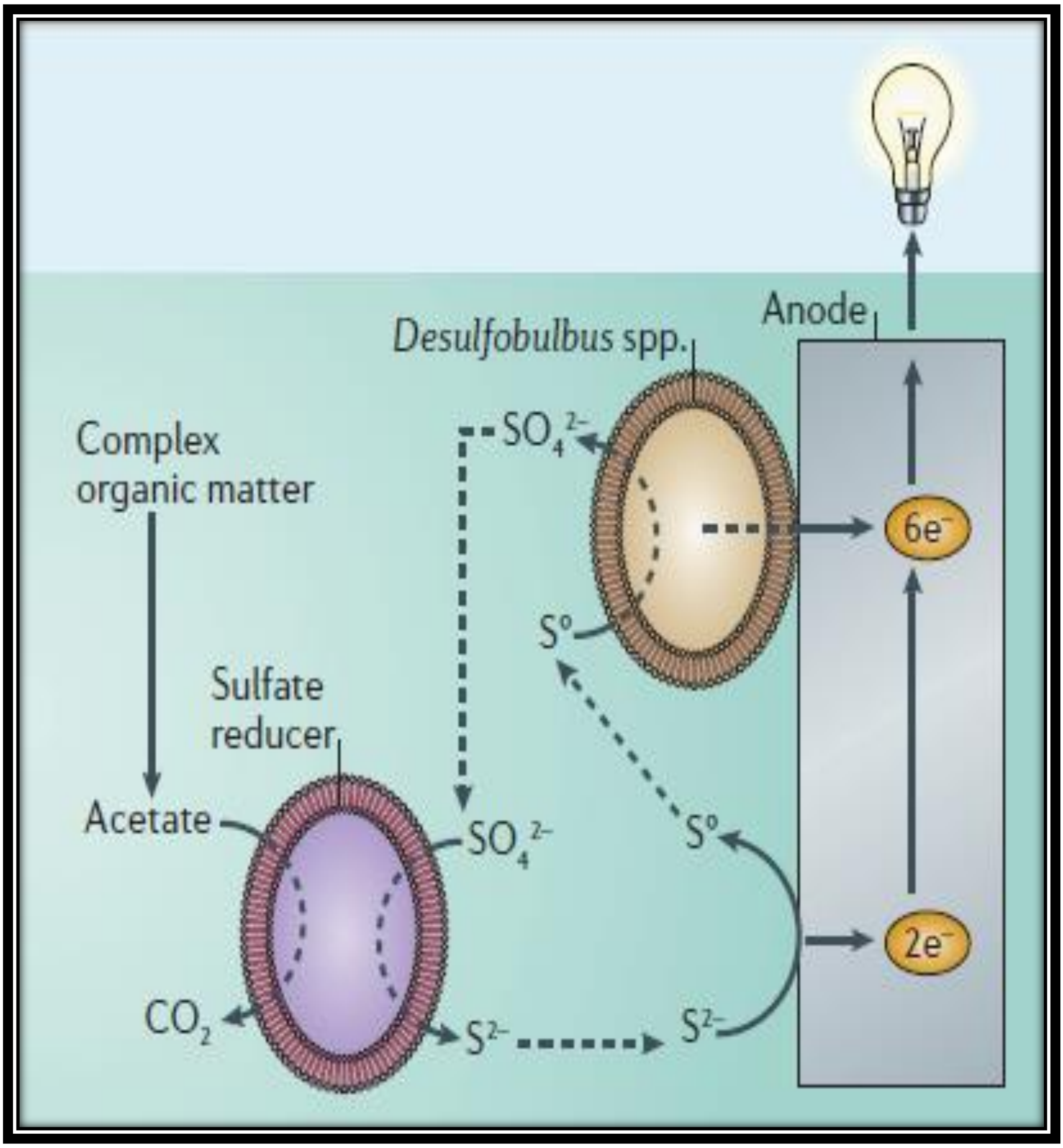

Fig 2: Mechanisms by which reduced sulphur compounds can contribute to electricity production

In sediment microbial fuel cells in sulphide-rich sediments. Sulphate (SO42–) reducers produce sulphide (S2–) which can abiotically react with the anode, yield 2 electrons and sulphur (S0). *Desulfobulbus* species that colonize the anode can oxidize S0 six additional electrons are extracted and recycled the sulphate.

Micro-organism	Substrate	Anode	Current (mA)	Power (mW/m^2)	Reference
Shewanella putrefaciens	lactate	woven graphite	0,031	0,19	Kim *et al.* 2002
Geobacter sulfurreducens	acetate	graphite	0,40	13	Bond and Lovley 2003
Rhodoferax ferrireducens	glucose	graphite	0,2	8	Chaudhuri and Lovley 2003
	glucose	woven graphite	0,57	17,4	Chaudhuri and Lovley 2003
	glucose	porous graphite	74	33	Chaudhuri and Lovley 2003
Mixed seawater culture	acetate	graphite	0,23	10	Bond *et al.* 2002
	sulphide /acetate	graphite	60	32	Tender *et al.* 2002
Mixed active sludge culture	acetate	graphite	5	-	Lee *et al.* 2003
	glucose	graphite	30	3600	Rabaey *et al.* 2003
	sewage	woven graphite	0,2	8	Kim *et al.* 2004

3.2Oxidation of organic matter with electricigens

Microbial fuel cells that are closest to practical application are the sediment microbial fuel cell and are also called as Benthic Unattended Generator or BUG. These BUGs produces current from the stored organic matter in aquatic sediments. The potential application of BUG is to power electronic devices such as monitoring equipments of oceans and other aquatic environments at the bottom.

An electrode is typically a plate of graphite which is embedded in anoxic sediments and serves as anode. When the anode is connected to graphite electrode which serves as cathode in overlying aerobic water, there is a flow of electron. BUGs operate by principles similar to microbial fuel cells with reduced end products of microbial metabolism, such as sulphide reacting with anode or by electron shuttling between microbe's andelectrodes with naturally occurring electron shuttles. The analysing of microbial community that colonize the surface of anode by 16rRNA genes characterization revealed that there was enrichment of microorganisms in *Geobacteracea*e family on anodes harvesting electricity from sediments. This enrichment on anodes harvesting electricity from fresh sediments and diversity of marine.

Pure cultures of *Geobacteraceae* oxidize the typical electron donors with transfer of electrons to electrodes and conserve energy to support growth.

Acetate is the most important electron donor as it plays central role in the organic matter degradation

By anaerobic microbial consortia. The complex organic matter in anaerobic sediments is degraded by consortium of fermentative microorganisms and *Geobacteraceae* when Fe^{3+} is electron acceptor.

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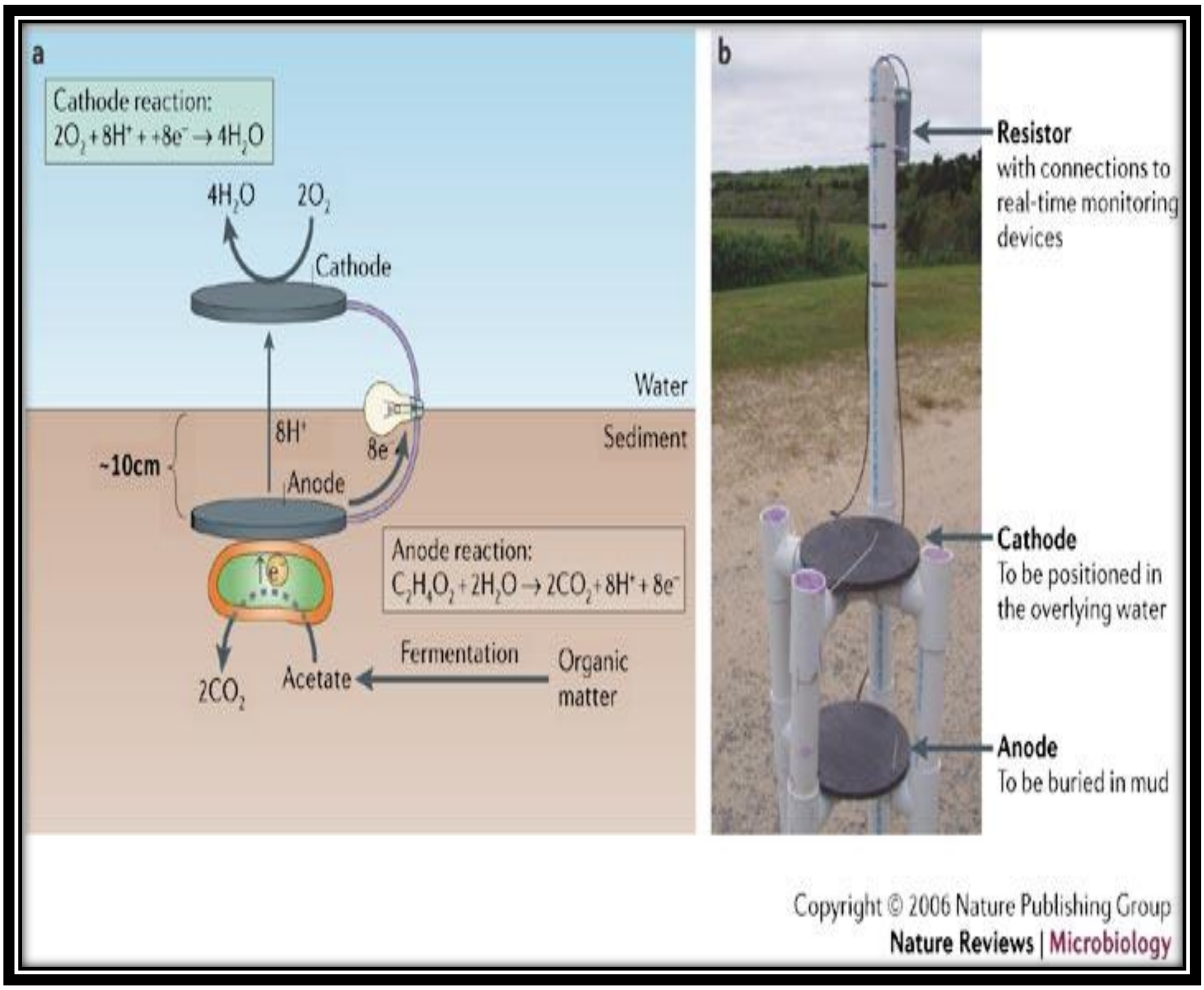

Fig 3: A sediment microbial fuel cell. A | A schematic of a sediment microbial fuel cell. Organisms in the family *Geobacteraceae* can oxidize acetate and other fermentation products, and transfer the electrons to graphite electrodes in the sediment. These electrons flow to the cathode in the overlying aerobic water where they react with oxygen. **B|** An actual sediment fuel cell before deployment.

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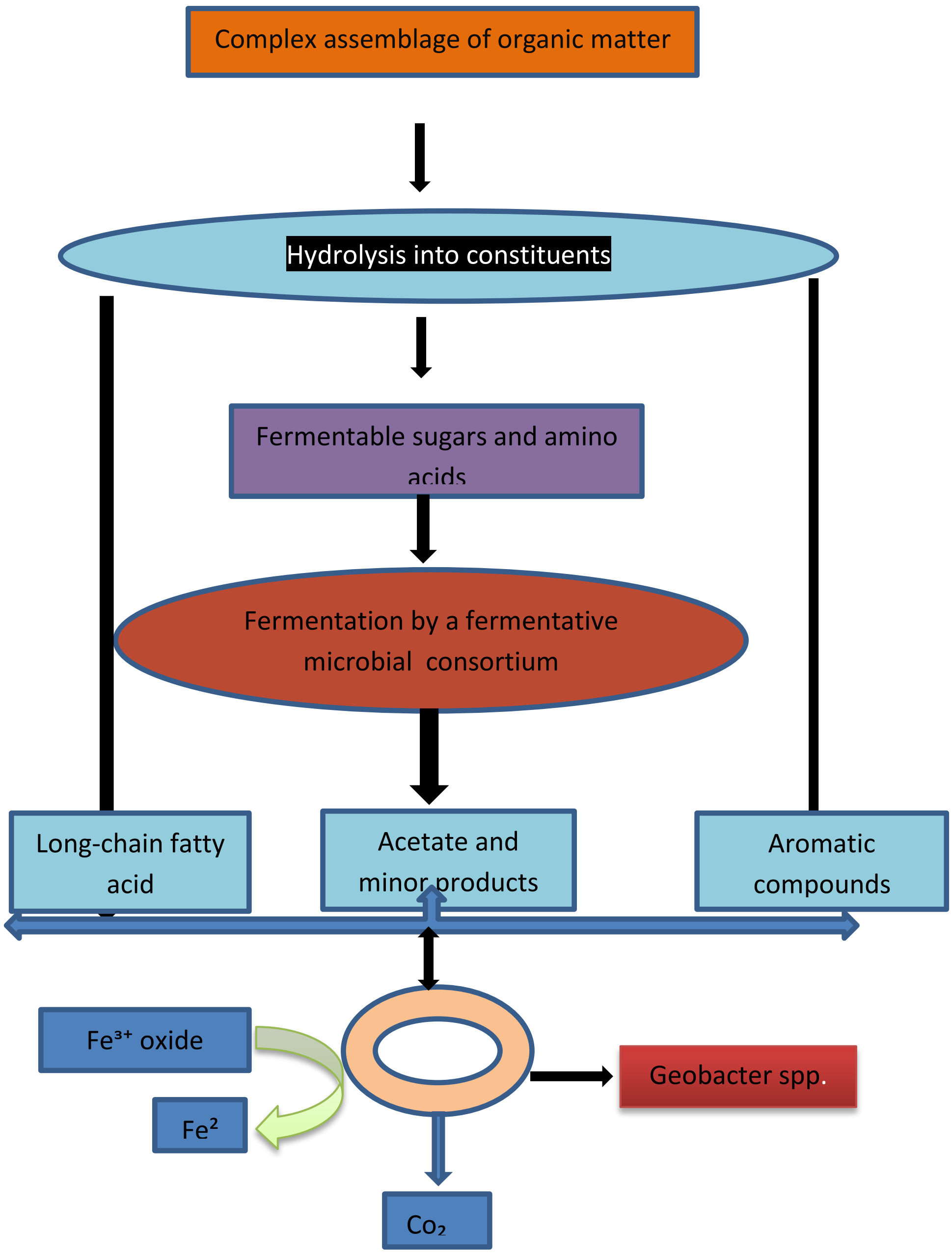
3.3 Liberation of electrons from organic matter

Anaerobic metabolism with current microbial fuel cell should be promoted at the anode to convert organic matter to electricity in an effective manner. For this type of metabolism of organic matter fermentation is a well-known mechanism and many microbial fuel cell studies relied on fermentative microorganisms. Most of the biomass and wastes requires the fermentation products from sugar, amino acid metabolism and related compounds. In addition to other constituents such as long-chain fatty acids, aromatic compounds are oxidized with electron transfer to an electron acceptor. Fe^{3+} oxides are the closest analogues to electrodes for microbial metabolism in natural environment because both electrodes and Fe^{3+} oxides are insoluble, extracellular electron acceptors. In sedimentary environments the oxidation of organic matter are coupled to the Fe^{3+} oxides reduction requires the cooperation of a consortium of fermentative microorganisms and Fe^{3+} reducing microorganisms. *Geobacter* species in temperate environments and Fe^{3+} reducing archea in hot environments (most often Fe^{3+} reducing microorganisms) metabolize the fermentation products and organic compounds so that fermentative microorganisms do not metabolize readily by oxidizing them to carbon dioxide with Fe^{3+} oxides serving as electron acceptor. In order to effectively convert organic matter to electricity similar consortia and pathways are required with exception that the anode serves as the final electron acceptor.

Fig 4: Generalized pathway for the anaerobic oxidation of organic matter to carbon dioxide with Fe3+ oxide serving as an electron acceptor in temperate, freshwater and sedimentary environments.



3.4 Mechanism of electron transfer to electrodes:

If there better understands of how electricigens transfers electrons to anode it will be more useful to design better anode materials to interact with appropriate electron transfer protein. It is important to recognize microorganisms that have optimized electron transfer to natural extracellular electron acceptors such as Fe^{3+} oxides unlikely there has been substantial evolutionary pressure to select most effective strategies for production of electricity. The ability of electricigens to produce electricity is related to their capacity to transfer electrons onto extracellular electron acceptors like Fe^{3+} and Mn^{4+} oxides as well as humic substances. One of the most awful barriers to microorganisms transferring electrons onto Fe^{3+} or electrodes is non-conducting lipid membrane system. It serves as an insulator, separating cytoplasm where electrons are extracted from organic matter during central metabolism from outside the cell where final electron transfer takes place. *G.sulfurreducens* transfers electrons that are derived from central metabolism onto extracellular Fe^{3+} oxides begin to emerge a series of C-type cytochromes associated with the inner membrane, periplasm and outer membrane might interact to transfer electrons to the outer surface of membrane. The presence of specialized pili is required for growth on Fe^{3+} oxides that are localized to one side of the cell. Pili are the electrical conduit between the cell and Fe^{3+} oxides.

G. sulfurreducens form close contact between the cells and anode, little more than a monolayer on the surface of electrodes. Under these conditions current can be produce in the absence of pili providing the cells to retain the ability to produce outer- membrane cytochromes, OmcS[68]. OmcS is displayed on outer surface of the cell as they are essential for Fe^{3+} oxide reduction. OmcS can make electrical contact with flat surface of electrodes, alleviating need for the conductive pili that seems to be required for the effective contact with heterogeneously dispersed Fe^{3+} oxides. In improved systems with a greater power output, thick, visually apparent biofilms are formed on anode. The conductive pili are essential for the development of thicker biofilms and high current production level. The pili are involved in electron transfer to the anode for cells and not in direct contact with anode surface.

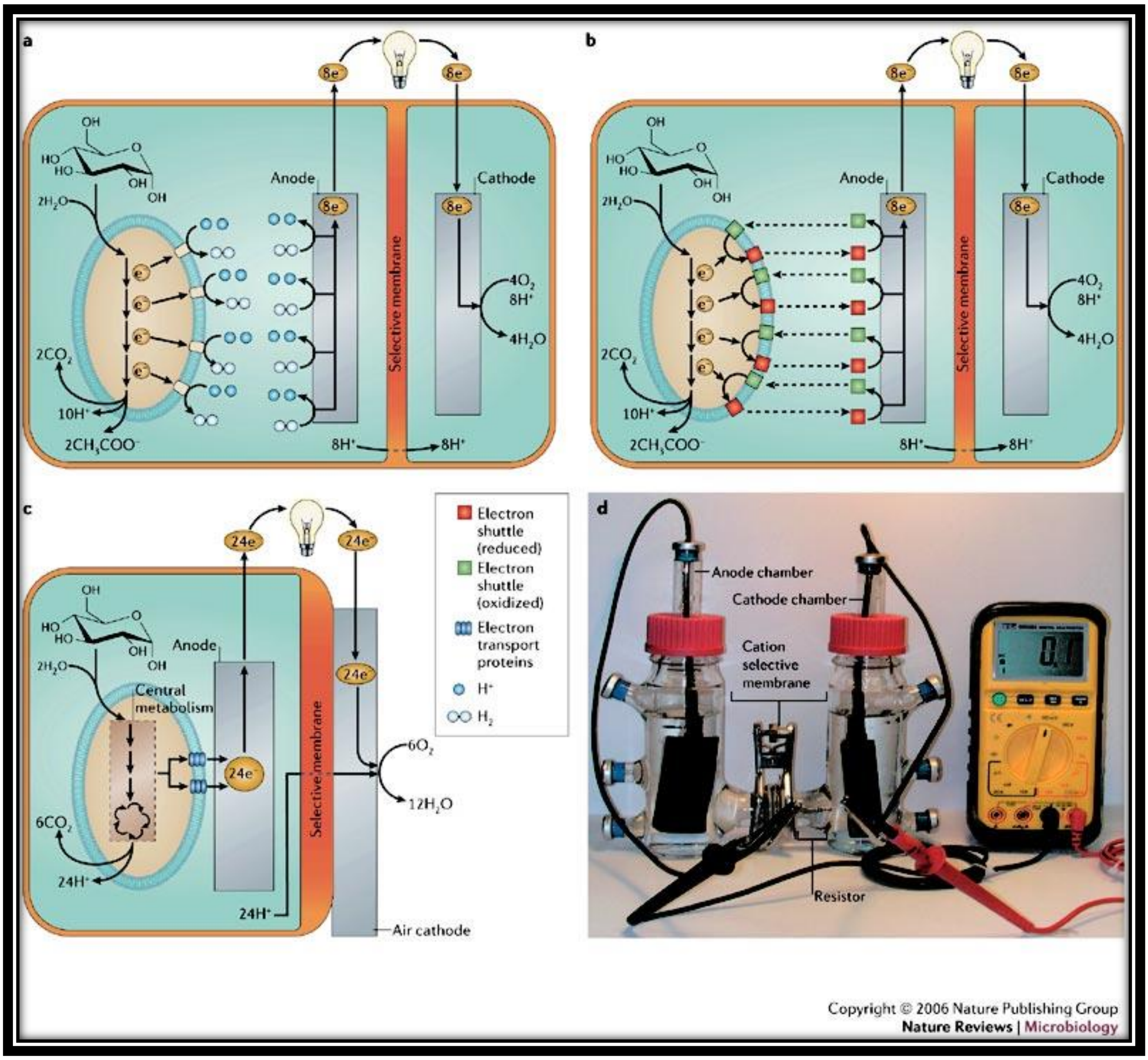


Fig 4: microbial fuel cells producing electricity through different mechanisms of electron transfer to the anode**.** Glucose serves as an example fuel. **A|** an indirect microbial fuel cell. A fermentative microorganism converts glucose to an end product, hydrogen, which can abiotically react with the anode to produce electrons and protons. This process only partially recovers the electrons available in the organic fuel as electricity, and results in the accumulation of organic products in the anode chamber. **B|** A mediator-driven microbial fuel cell. An electron-shuttling mediator accepts electrons from reduced cell constituents and abiotically transfers the electrons to the anode. The reoxidized mediator can then undergo repeated cycles of reduction and oxidation. In most instances, the cells that have been used in such fuel cells only incompletely oxidize their organic fuels as shown **.C|** The oxidation of glucoseto carbon dioxide with direct electron transfer to the electrode surface. Glucose is taken into the cell and oxidized tocarbon dioxide by typical central metabolic pathways, such

as the tricarboxylic acid (TCA) cycle. Electrons derived from glucose oxidation are transferred across the inner membrane, periplasm, and outer membrane through electron transportproteins, such as c-type cytochromes. In this example, the system is illustrated with an air cathode rather than a cathode submerged in water.

D| A two-chambered microbial fuel cell. This system is not optimized for maximum power production but is convenient for microbiological studies

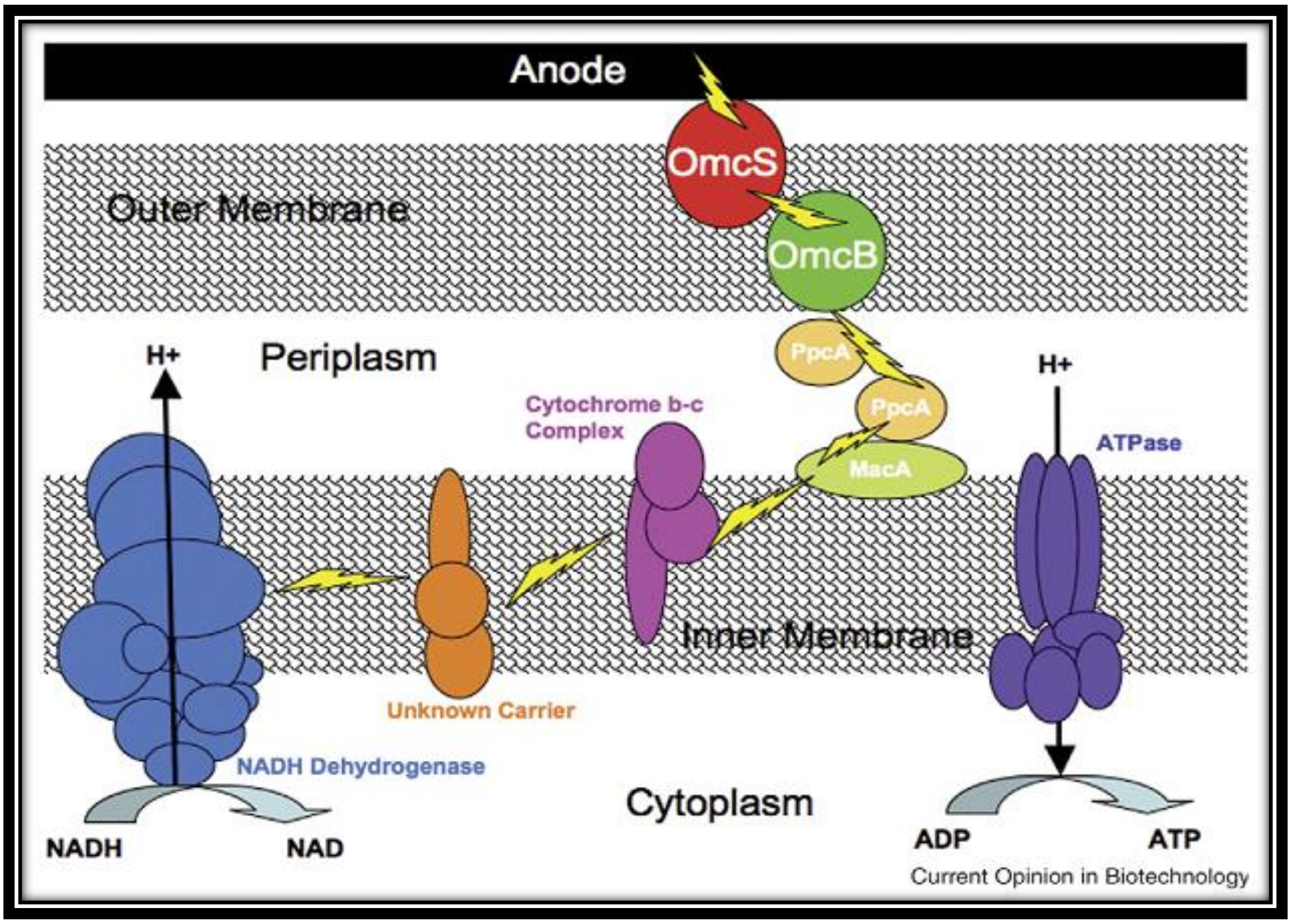


Fig 5: A mechanism for extracellular electron transfer by *Geobacter sulfurreducens*.**A|** A transmission electron micrograph showing the association of Fe3+ oxide (indicated by arrows) with pili expressed by *Geobacter sulfurreducens*.The inset shows pili that are intertwined with Fe3+ oxides. **B|** Potential route for electron transfer to Fe3+ oxides by *Geobacter sulfurreducens*. This model is based on a previous model16 and subsequent findings41, 69. MacA, PpcA, OmcB,OmcE and OmcS are c-type cytochromes which genetic studies have indicated are required for optimal Fe3+ reduction.The proposed electron flow between the cytochromes is based on their reported location within the bacterial cell. It is important to note that the genome of *G. sulfurreducens* contains genes that encode

approximately 100 c-typecytochromes 64, some of which might also participate in this electron transfer process. MQH2, menaquinol; MQ,
Menaquinone. Panel (**a**) is reproduced with permission from Nature REF. (2005) .

4 Nanowires:-

Nanowires are the electrically conductive appendages produced by a number of bacteria most probably from the *Geobacter* and *Shewanella*. These wires constitute a complex nanoweb structure between the anode surface and the microbial community. The nanowires are of, only 3-5 nanometers in width, quite durable and more than thousand times long as they are wide. Nanowires are required for the long- range extracellular transfer of electrons. *G. sulfurreducens* produces cytochromes .The genetic studies and gene expression analysis suggests that the outer surface cytochromes OmcE and OmcS are involved in electron transfer to the anode surface. The species of *Geobacter* produces fine, hair like structures known as pili. Pili are thin and long, conductive structures and conduct the flow of electrons to the solid minerals. *Geobacter sulfurreducens* nanowires are type IV pili that are composed of pilin protein structural subunit Pili. Pili play an important role in relieving electron acceptor limitations, by increasing the cell surface are for localization of cytochrome and storage of electron in order to periplasmic reoxidization and inner membrane electron carriers (Strycharz- Glaven *et al*. 2011).In the oxygen absence, a cell needs to find a way to transfer the electrons outside the cell membrane and this is what nanowires exactly do. Nanowires produced by *Shewanella oneidensis*MR-1 also exhibit nonlinear electrical transport.

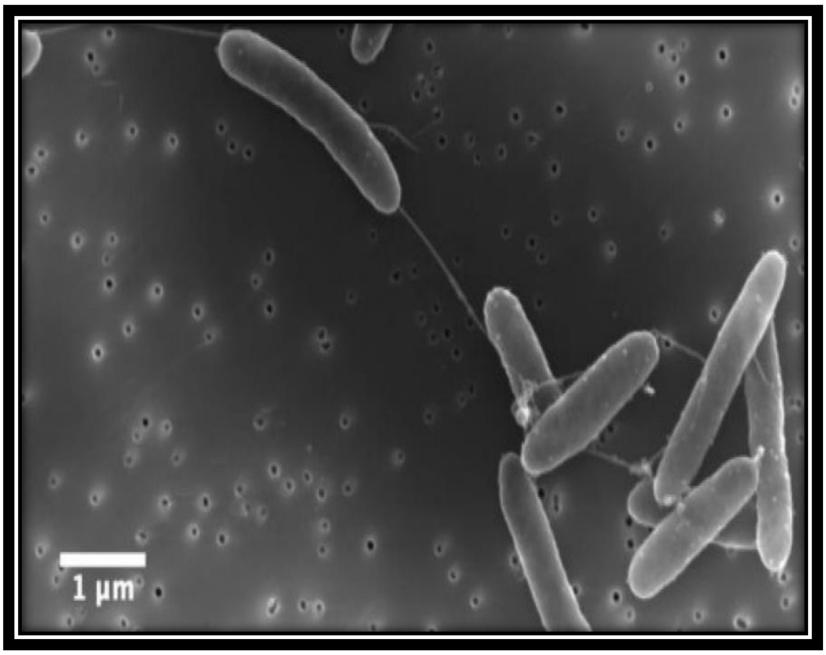


Fig 6*:* *Geobacter* *sulfurreducens* Cacao et *al*.1995

5 How Do MFCs Work?

Microbial fuel cells work by allowing bacteria to grow by catalysing chemical reactions and harnessing and of energy is stored in the form of ATP (adenosine triphosphate). MFCs allow the bacteria to oxidize and reduce organic molecules. Bacterial respiration is a redox reaction in which the electrons move around. In some bacteria, substrates are reduced, oxidized and transfer of electrons through respiratory enzymes by NADH. A MFC consists of anode and cathode separated by a specific membrane. At the anode microbes oxidize organic fuel and generate protons which pass through the membrane to cathode, while the electrons which pass through the anode to an external circuit generate current.

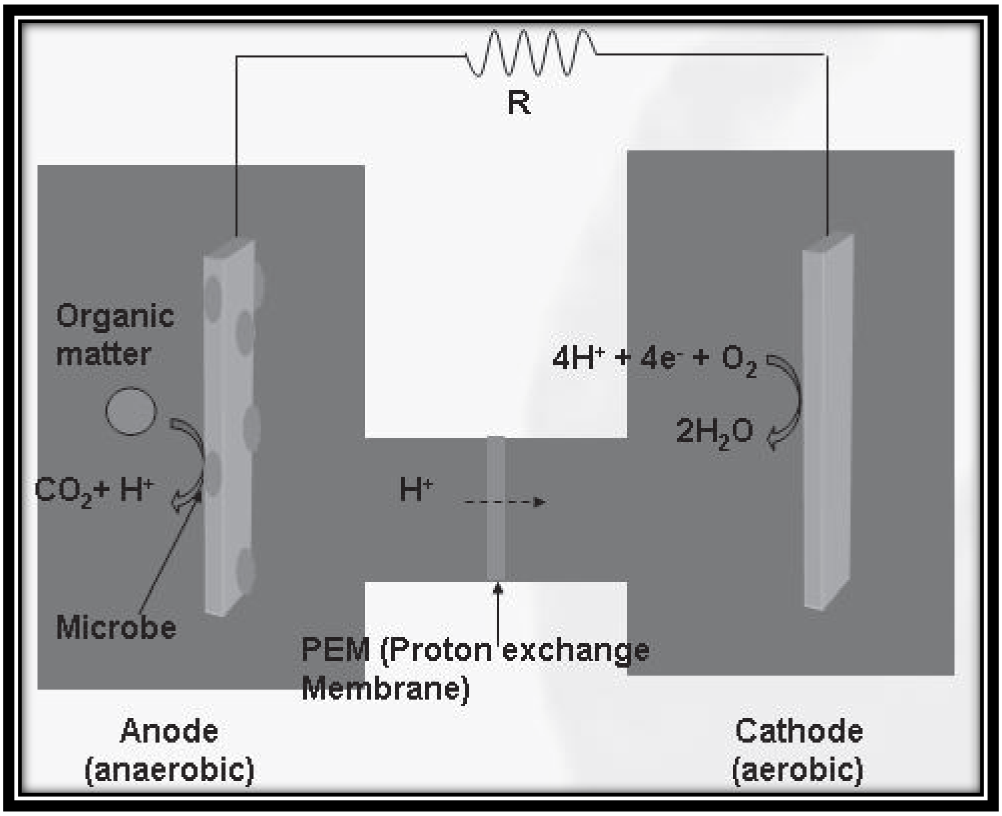


Fig 7: Microbes remove the electrons from organic matter and transfer them to the anode in the anaerobic chamber. The electrons move across the resistor to the cathode where they combine with protons, and oxygen to form water. (Figure courtesy of Jung Rae Kim)

MFC works in three ways:

- Electron transfer by mediators
- Direct electron transfer through bacteria outer membrane enzymes
- Electron transfer via pilus-like nanowires

A. **Electron transfer by mediators-**

Pseudomonas aeruginosa is one of the dominant species in fuel cells. Pseudomonas is a facultative anaerobic bacterium, which generally only use oxygen or nitrate as terminal electron acceptor. *Pseudomonas aeruginosa* and several Pseudomonas species are described for their production of phenazine and phenazine derivatives. One of the most common phenazine derivatives is Pyocyanin which plays an important role in electron transfer from bacteria to electrogens

B. Direct transfer of electron through bacteria outer membrane enzymes-

Microorganisms transfer electrons to the anode by direct electron transfer from the respiratory enzymes (cytochromes) to the electrode (Gil *et al*., 2003). This lead to development of new type of MFCs i.e mediatorless (B.H. Kim et al., 1999). The Fe (III) reducer *Shewanella putrefaeciens, and Geobacter sulfurreducens* bacteria are electrochemically active. These bacteria transfer electrons extracellularly and are known as exoelectrons. These mediators trap electrons from the respiratory chain and reduce to transfer electron to the d through outer cell membrane (Bond *et al*., 2002). Bacteria like *Desulfobulbus propionicus* (Holmes *et al*., 2004) *Shewanella putrefaciens* (Kim *et al*., 2002), *Geobacter sulfurreducen* (Bond and Lovley, 2003) generate electricity in mediatorless MFC (Jang *et al*., 2004).

C. Electron transfer via pilus- like nanowires

Nanowires are produced by *Shewanella oneidensis* MR-1 that exhibit non-linear electrical transport properties and use exocellular electron transfer which results in generation in MFC. *S. oneidensis* produces flavins that function as electron shuttles.

*Shewanella oneidensis*MR-1 (*25*) directly involved in electron transfer extracellularlly. *S. oneidensis* results in poor conductive nanowires, loss of electrochemical activity, and loss of the ability of reduction of insoluble electron acceptors. These nanowires allow the reduction of distant acceptor of electron and removes the need for soluble mediators that would lost in a continuous-flow MFC and direct interspecies electron transfers.

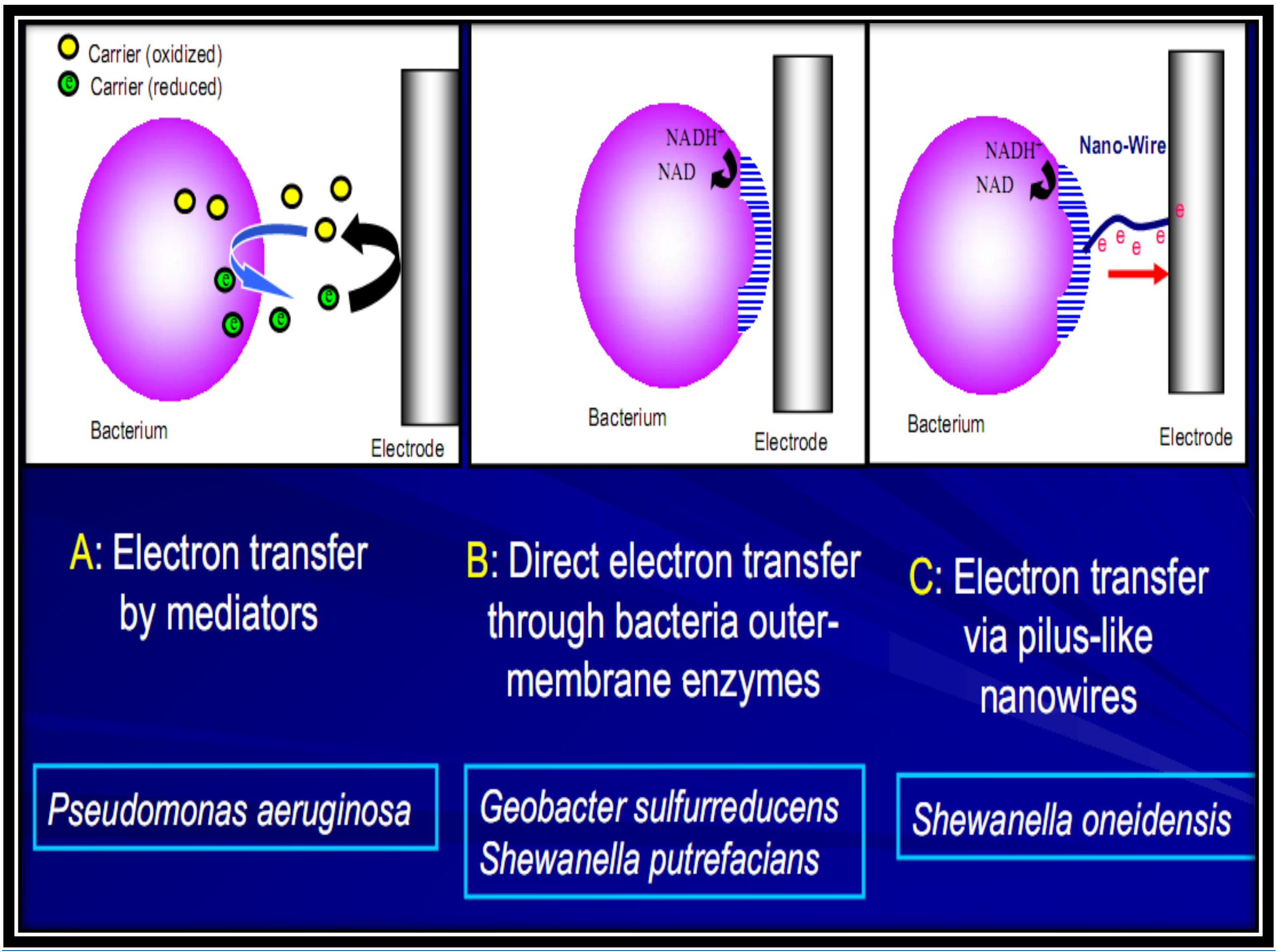


Fig 8 Electron transfer by bacteria

(www.wikipedia.com)

5.1. Extracellular electron transfer to the anode

In an MFC, to enable the cellular respiration and conversion of substrate to CO_2 the electrons have to be transferred from cellular metabolism extracellularly to the electrode. There are several mechanisms to carry out this function.

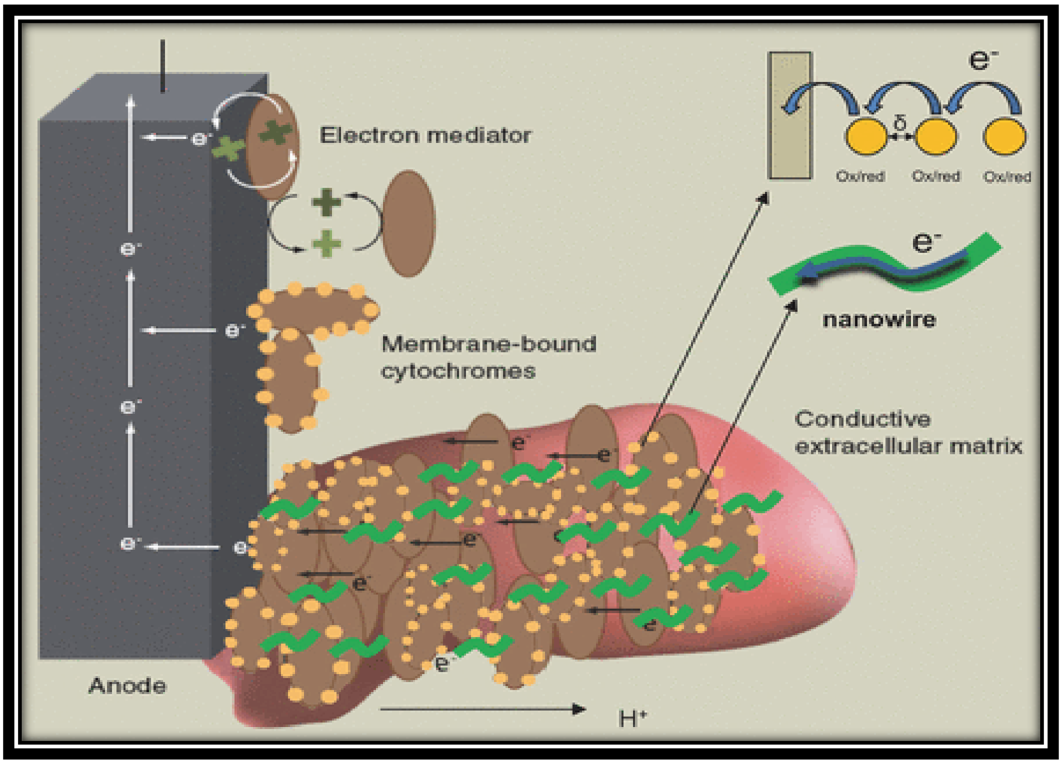


Fig 9; - Methods for extracellular electron transfer to an anode acting as an electron acceptor in a microbial fuel cell.

An electron mediator is reduced by microorganisms and oxidized at the electrode surface (electron mediator). Microorganisms may directly transfer electrons to the electrode surface via membrane bound proteins such as cytochromes (membrane bound cytochromes). Some organisms have been reported to be capable of long-range electron transport through a conductive extracellular biofilm matrix. The mechanism of long-range transport through such a conductive matrix is unknown but may involve cytochromes and/or microbial nanowires.

In earliest studies it is demonstrated that fermentative organisms could generate an electrical current in MFC. It is suggested that electrons may be transferred to the electrode through the direct interaction of reduced fermentation products. With the addition of artificial electron mediator's power output gain in MFCs were observed. These electron shuttles are the compounds that are capable of transferring across the cellular membrane and accepting electrons from one or more electron carriers within the cell. The compound will be transferred out of cell when reduced and oxidized on the anode surface there is shuttling of electrons from central metabolism to the anode. On addition of mediators to the MFC some bacteria, species are reliant to allow the transfer of electrodes to the anode such as 2,6-dichlorophenolindophenol, thionine, thoquinone, benzylviologen, 2-hydroxy-1,2-naphthoquinone and various phenazines, phenothiazines, iron chelates, phenoxoazines,and neutral red. As organic compounds generally not reduce fully to carbon dioxide, these systems are inefficient. Proteus vulgaris is one exception which is able to reduce sugars completely to carbon dioxide using thione for electron transfer.

Some bacteria like *Pseudomonas spp*, *Geothrixfermentans*, *Shewanella spp.* are able to produce electron mediators. Mediators are energetically expensive for bacteria to produce and must be cycled many times for bacterial species to recoup the metabolic cost. Growth of cells in the system are allowed by mediators which require the mediator to diffuse through the entire culture and decreasing the efficiency of current production and causing slow grown cells to be lost in flow through systems. There are several bacteria that can transfer electrons directly to anode surface without the need of mediator by a biofilm formation and interacting directly with the anode surface. It was found that *S.putrefaciens* MR-1 transfers the majority of extracellular electrons through flavins, which acts as electron mediator. The other organisms like *Aeromons hydrophilia* *Shewanella* spp,, *Clostridium* spp., *Rhodoferax ferrireducens*, *Desulfobulbus propionicus* and *Geobacter spp.* are capable to transfer electron directly to an anode surface.

(Biofuels (2010).

6...Electricity production from treatment of urban waste water using MFC

The biological fuel cells can convert chemical energy of organic matter directly into electricity. Number of biological fuel cells is there that include

i. Processes that use primary fuel usually organic matter such as corn husks, urban waste water, whey etc to generate hydrogen or ethanol which then are used as a secondary fuel within a conventional fuel cell.

ii. The cells which generate electricity directly from organic fuel such as glucose that use either enzymes or complete microorganisms, electron mediators are oftently need to transfer electrons from microorganisms to electrode.

iii. Cells that combine the utilization of photochemically active systems and biological moiety to harvest energy from sunlight and convert it into electricity.

MFCs consist of fuel cells in which bacteria directly catalyze the conversion of organic matter into electricity without addition of mediators or artificial electron shuttles. Both one and two chamber MFCs are reported to obtain higher efficiencies. The power density produced by MFC is low and normally below $50Wm^{-2}$. Hence they are considered to have more potential of commercial application than some other kinds of biofuel cells due to their simplicity. They do not require artificial electron shuttles which are expensive and toxic to microorganisms. In this MFC is fed with urban waste water as a fuel. This method is proposed to rapidly generate microorganisms' culture that is capable of producing electricity from waste water.

6.1. Experimental set up

Domestic waste water is collected from primary clarifier of the Ciudad Real Wastewater Treatment Plant (WWTP). The main element of this system is biological reactor or anolyte chamber where microorganisms remove the organic material and produce electricity. The reactor consists of a glass cylindrical chamber (Alamo, Spain) of an empty bed of volume $1000cm^3$. The anode consist of a graphite cylinder placed with a total surface of 20 cm^2 placed in the reactor. The cathodic chamber of volume 100 cm^3 is connected with anodic chamber through salt bridge. The cathode consists of porous graphite bar of 20 cm^2. The set up works in continuous mode. A peristaltic pump fed continuously at a flow rate of

0.34 dm^3 h^{-1} of actual urban wastewater (effluent of the primary decanters of the municipal WWTP of Ciudad Real) to the chamber of anode. A compressor of fisheries (maximum flow-rate of 0.34 dm^3 min^{-1} and maximum pressure of 1.2m of water column) connected to the porous cathode to supply oxygen to the cathodic chamber. In normal operation cathode and anode were connected through wires and a resistance. A keithley 2000 Digital Multimeter was connected to the system for continuously monitoring the value of the cell potential. Polarization curves are recorded using Autolab PGSTAT30 galvanostat/potentiostat (Ecochemie, The Netherlands). Impedance spectra are being recorded by the frequency response analyzer (FRA) through operating MFC by using Autolab PGSTAT30 module .Chemical oxygen demand (COD) is determined by the use of pH and HACH DR2000 analyser, conductivity and oxygen dissolved are measured by means of GLP22Crison pH-meter, LF538 WTW conductivity- meter, Oxi538 WTWoxy-meter respectively.(M.A. Rodrigo∗, P. Ca˜nizares, J. Lobato, R. Paz,C. S´aez, J.J. Linares Department of Chemical Engineering. *Castilla-La Mancha* University*, Campus Universitario s/n, 13004, Ciudad Real, Spain*Available online 31 January 2007).

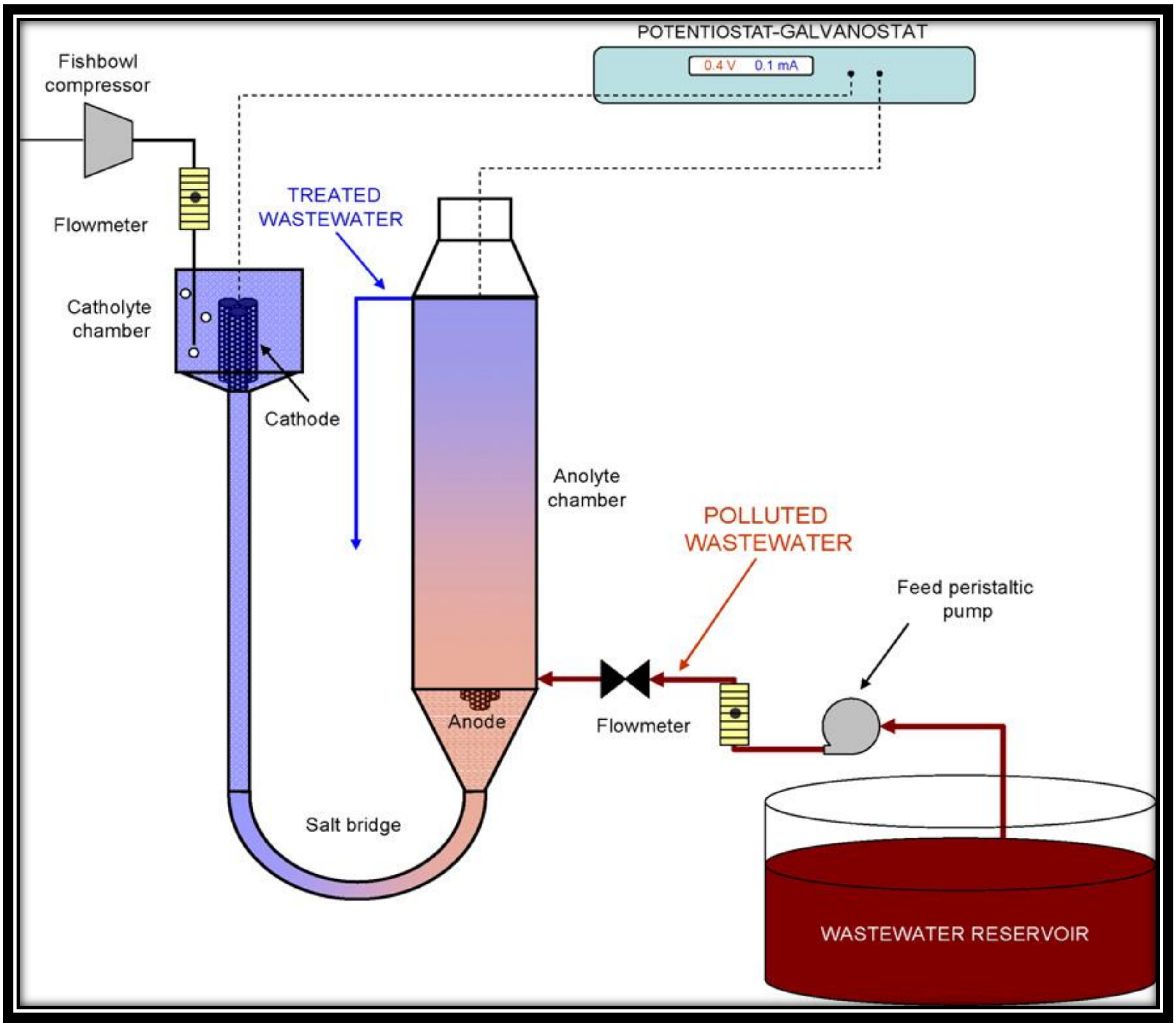


Fig:Microbial fuel cell setup

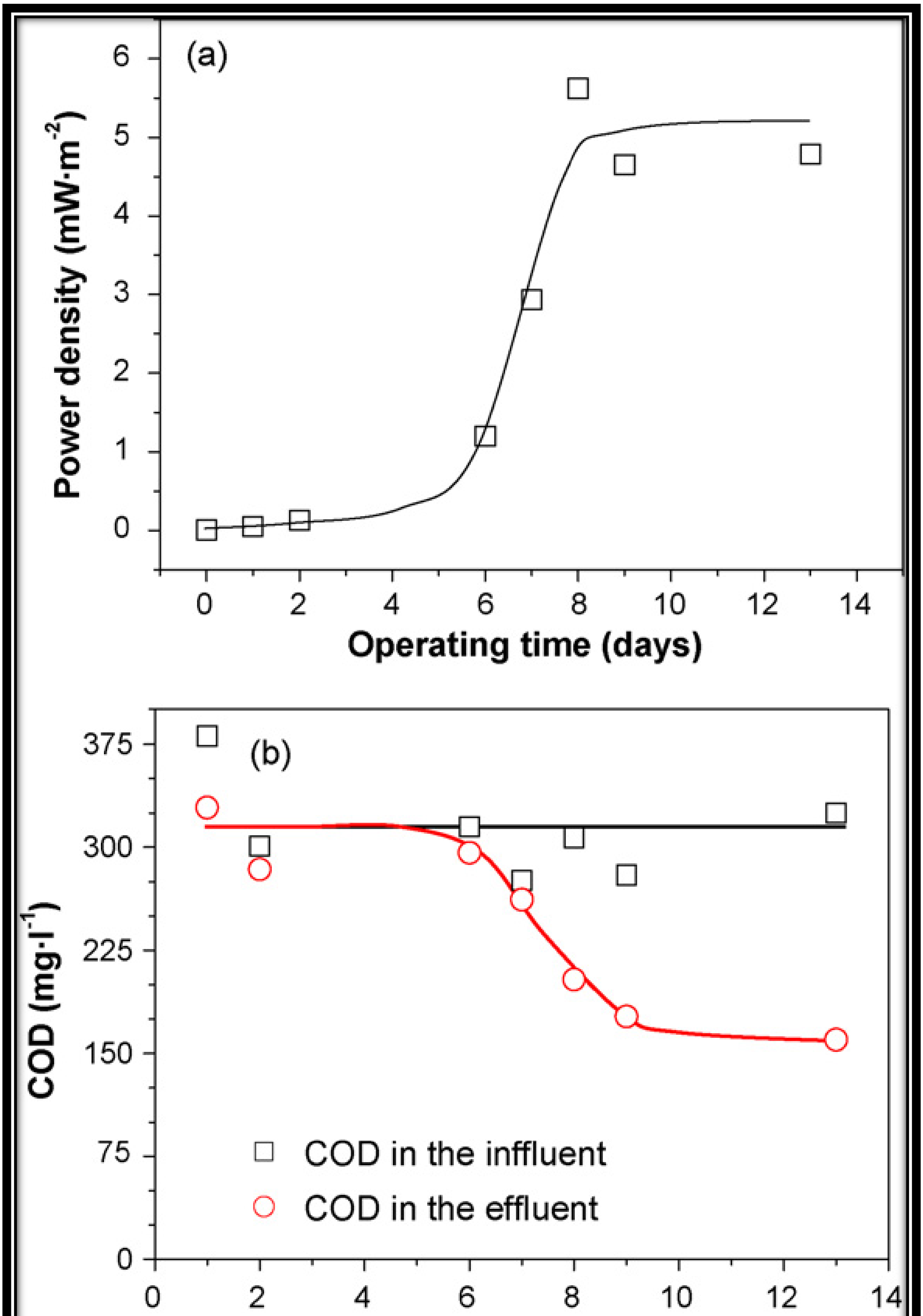
6.2. Results and Discussion

Conditioning stage-

To start the process the obtained activated sludge in the biological reactors of municipal wastewater treatment plant of Ciudad Real (Spain) placed in a closed-tank without aeration formation of a mixed culture of aerobic and anaerobic microorganisms is done during 5 days. Wastewater is not fed to the system during conditioning period, so only substrate is available for microorganisms was coming from endogenous metabolism.

After that the sludge is placed in MFC and anodic chamber is fed with actual urban wastewater obtained in the WWTP of Ciudad Real (collected after primary decanter) and the air is left to flow through cathode. Concentration of oxygen is measured in both anodic and cathodic chambers. By these measurements we are unable to confirm the concentration of oxygen in the anolyte is zero so anodic chambersis considered as anaerobic.

The wastewater feed contains all the soluble pollutants of raw wastewater and suspended solids in small quantities. A resistance of 125Ω is placed to an external circuit i.e between the anode and cathode. The increase in power with time is observed until obtaining steady-state conditions and the acclimatization period is shorter than 10 days. The density of the operation steady-state power is around $5mWm^{-2}$ (125Ω resistance) and us inside the typical range of values. The biological culture is capable of generating electricity from urban wastewater is not a time-consuming process. Efficiency of the treatment is improved during conditioning process and steady-state in the effluent COD reach for the values close to 150mg dm^{-3}. By the change in operation conditions this value should decrease. Only a small percentage of COD is removed by the electricity generating process. At the steady-state conditions power density obtained is around 24mW m^{-2}. The value obtained at a cell potential is of 0.23V. The potential of open circuit increased with time. The value of steady-state of this parameter is 0.42V.


(a)
Power density (mW·m-2)
6
5
4
3
2
1
0
0
2
4
6
8
10
12
14
Operating time (days)
(b)
COD (mg·l-1)
375
300
225
150
75
0
0
2
4
6
8
10
12
14
COD in the inffluent
COD in the effluent
Operating time (days)

7. Electricity generation from potato wastewater in MFC

MFC studies use pure compounds such as acetate (Bond &Lovley, 2003), sucrose (He *et al*., 2006), glucose (Rabaey*et al*., 2003), an amino acid that is cysteine (Logan *et al*., 2005), or a protein i.e. bovine serum albumin (Heilmann *et al*., 2006). In MFC tests the waste water sources that have been utilized including a domestic wastewater (Liu *et al*., 2004), , food processing wastewater (Kim *et al*., 2004), swine wastewater (Min *et al*., 2005), hydrogen fermentation reactor effluent (Oh and Logan, 2005), and corn stover hydrolysates i.e. liquefied corn stover (Zuo*et al*., 2006) and paper industry wastewater (Mathuriya and Sharma, 2009). MFC technology can use the bacterium that are present already in wastewater for electricity generation and simultaneously treating wastewater (Lui*et al*., 2004; Min and Logan, 2004).When wastewater is used as fuel, biofilm formed onto the anode in addition to microbial clumps which are loosely associated with electrode. The microbial clumps ferment the complex fuel into simple fermentation products which are then oxidized by electrochemically active microorganisms in bio-film (Kim *et al*., 2002; Bond and Lovely, 2003).The benefits of using MFCs for wastewater treatment are clean, low emissions, safe, quiet performance, higher efficiency and direct electricity recovery.

The potato is starchy and tuberous crop of *Solanaceae* family. It contains all nutrients required for perfect microbial growth. There are large to small scale industries that are involved in potato progressing (Jai Gopal andKhurana, 2006; Hijmans and Spooner, 2001). Potato wastewater has high Chemical Oxygen Demand (COD) but its non-toxic because most of the organic matter that are present in water consists of sugar, starch, and protein. Potato wastewater is suitable for electricity generation due to lack of high inhibitory substance concentration and its food derived nature.

have been conducted showed electricity generation by the use of MFCs, after two cycles stable current output was achieved.

8. Fuel cells applications

8.1 Waste water treatment

It is the most important foreseeable application of an MFC is waste water treatment. Exoelectrogens breakdown and metabolize the carbon rich sewage of waste water stream for the production of electrons that stream into a cheap conductive carbon cloth anode. An MFC is used in the treatment system as a replacement for existing energy demanding bioreactor resulting in net energy- producing system. MFC based system provide an opportunity for better removal of BOD and nutrients. Applications of MFC are particularly useful in such areas where septic tanks cannot be used because of the need of high BOD removal.

8.2 Environmental sensors

MFCs can be used as a convenient biosensor for waste water stream to power devices particularly in river and deep-water environments where it is difficult to routinely access the system for replacement of the batteries. The sediment fuel cells are developed to monitor environmental systems such as rivers and ocean.

Fig 9: Sediment microbial fuel cell

8.3 Bioremediation

In bioremediation system the MFC is not used to produce electricity instead of it power can be put into the system for desired reactions to remove or degrade chemicals like converting soluble U (VI)to insoluble U (VI). In this the bacteria not only donate electrons to an anode but also accept electrons from cathode. The uranium is precipitated onto a cathode due to bacterial reduction by poising the electrodes at -500mV. (Gregory *et al*. (40)). When electrodes use as electron donors then nitrate can be converted to nitrite.

8.4 Hydrogen Production

By the removal of oxygen at the cathode and adding it in a small voltage via bioelectrochemically assisted microbial reactor (BEAMR) process or the biocatalyzed electrolysis process the MFCs can be modified for the production of hydrogen gas(H_2). This can be done by keeping both chambers anaerobic and supplementing the MFC with 0.25 volts of electricity because of overpotential cathode.

9. Conclusion

MFCs are evolving to become robust and simple technology for renewable energy production. The study of MFCs documents the feasibility of bioelectricity generation from waste water treatment with anode materials without any toxic mediators. This biological process is not very time consuming. They are useful in specialized applications as BOD sensing, powering underwater monitoring devices, hydrogen production, wastewater treatment, remote sensors, environmental bioremediation etc. The observed COD removal efficiency in anode chamber enumerates the functioning of MFCs as wastewater treatment unit in addition to renewable energy generation. These procedures are cost effective and environmentally sound and sustainable by the use of wastewater as substrate. Microbial fuel cell technology may qualify as a new core technology for the conversion of carbohydrates to electricity in years to come. The diverse range of bacteria is able to function and persists in an MFC to truly fascinate occurrence and understanding the knowledge of microbial ecology of biofilm and bacteria.

(Korneel Rabaey and Willy VerstraeteVol.23 No.6 June 2005), (Logan, B. E.; et al. Microbial Fuel Cells: Methodology and Technology. *Environ. Sci. Technol.(* 2006)

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