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Microbial Fuel Cells (MFC) and microalgae; Photo Microbial Fuel Cell (PMFC) as complete recycling machines

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Humans can exploit natural processes by microorganisms by using Microbial Fuel Cells and integrated Photo Microbial Fuel cells (MFC/PMFC) chambers containing electrodes to maximise microbial oxidation rates and rapidly recycle mass and elements at the quickest possible rates by control over both the microbes (choice of algae and bacteria) and the applied physicochemical conditions. This review focuses on natural recycling of essential elements by microbes, the productivity of bacteria and micro-algae as a fuel, decomposition and the use of microbial fuel cells to integrate both primary biomass production (in the cathode) with its decomposition and transformation by heterotrophic microbes (at the anode). The review discusses the potential future uses of photomicrobial fuel cells as complete recycling machines with advantages over all other biological recycling systems and these include rapid re-cycling rates, production of water, removal of carbon dioxide, evolution of oxygen, and the generation (rather than utilisation) of electrical power.

Introduction:

As the consequences of global warming continue to affect the climate, there is an increased need for new technologies that decrease dependence on fossil fuel consumption and promote sustainability.

Overpopulation along with over consumption, where resource use has outpaced the sustainable capacity of the ecosystem, are primary factors affecting the severity of all the other potential threat issues including global warming/climate change, biodiversity loss, desertification, deforestation, habitat destruction, ocean acidification, water pollution, waste (and its disposal), and resource depletion. The pressure to replace fossil fuels as soon as possible is building, but at the cost of introducing many more alternative power systems such as wind, hydro-, solar and combustible biomass. None of these conventional technologies,however well developed, can by themselves directly transform chemicals or recycle water or chemical elements in a useful way, and large scale conventional water treatment technologies are dependent upon the use of large amounts of electricity in order to function (e.g for purposes of pumping, mixing, aerating, filtration, ozonolysis, electro-osmosis or standard electrolysis for producing peroxide or bleach). What is urgently required is a technology that can efficiently clean up wet organic waste streams using the chemical energy contained within the waste itself to energise the rest of the system and therefore

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to recognise that all such wet organic material may be seen as a fuel, rather than being a negative value waste. The technology is one which oxidises reduced organic substrates by electron abstraction using anodic electrodes; an old technology [1] now brought up to date in the form of modern microbial fuel cells.

Nature's food chains, productivity and re-cycling by bacteria and microalgae

Regarding the productivity of lifeforms in nature, both bacteria and microalgae play the most important roles and make the highest contribution to the planet as a whole for recycling life's essential elements, Carbon, hydrogen, Oxygen, Nitrogen, Phosphorus and Sulphur (CHONPS). Primary biomass is produced by a group of life forms called autotrophs which make their own organic molecules. Primary producers (usually plants and other photo-synthesisers) are the gateway for energy and matter to enter food chains or webs. In general, the smaller the size of the particular living species (at the multi-cellular or unicellular levels of complexity) then the faster the growth rate and productivity can be. Oak trees are less productive than grass species, and elephants "grow" much more slowly than mice [2]. The same is true at the unicellular level for free living single celled microbes [3]. The smallest photosynthetic cell species (microalgae) grow much faster than any other type of plant, but this is still relatively slow compared to heterotrophic bacteria (a decomposer or consumer), the fastest of which is thought to be *Vibrio natriegens*, which (at NTP) has a mean generation time (mgt - the average time that a cell splits into two) of as little as 10 minutes [4] (i.e. μ=4.17h^{-1}). This growth rate should be compared to the equivalent of 1-2 days for most species of microalgae. For example, the mean growth rates of eight different cyanobacteria and eight different chlorophytes at 20°C, were 0.42 d^{-1} [0.017 h^{-1}] and 0.62 d^{-1} [0.026 h^{-1}] respectively, but growth rates were very similar at higher temperatures, between 0.92-0.96 d^{-1} at 29.2°C [5]. Comparison of *Synechocystis* sp. PCC 6803 with the green algae *Chlorella sorokiniana* 211-8K showed that the calculated biomass yield on light in continuous culture experiments, gave nearly equal values for both species [6]. The fastest natural photosynthetic cyanobacterium that has been reported [7], is thought to be *Synechococcus leopoliensis* UTEX 625 with a growth rate (μ) close to 0.1h^{-1} [2.4 d^{-1}] and with a corresponding mgt of 6.93h, which although fast for an algae is some 40 times slower than the heterotrophic *V. natriegens*.

Food chains and food webs:

A food chain is a linear sequence of organisms through which nutrients and energy pass as one organism eats another. In nature's food chains, each organism occupies a different trophic level, defined by how many energy transfers separate it from the basic input of the chain. There can be as many as 5 or 6 trophic levels. Food webs consist of many interconnected food chains and are a more realistic representation of consumption relationships in ecosystems. It is well known that the energy transfer between trophic levels is inefficient—with a typical efficiency of around 10% at each step of the chain. However, this is not the case for some organisms (Trophic level zero) that are categorised into detritivores; scavengers or decomposers of which the most important by far are the microbial (i.e. prokaryotic) decomposers, since their efficiency is much higher than 10%, and many species of heterotrophs and Archaea have high growth rates [8]. Heterotrophic prokaryotes, both bacteria and archaea represent the major living biomass in the oceans and play a vital role in marine food webs [9,10]. Their secondary production or bacterial production, resulting from the consumption of labile dissolved organic matter, represents a key pathway in the transfer of matter and energy to higher trophic levels [11]. The amount of carbon transferred ultimately depends on the amount and quality of organic matter and the composition of the microbial communities involved. It has been estimated that about half of the ocean's total primary biomass production has been processed by microbes [12]. In the simplest scheme, the first trophic level (level 1) is plants (primary producers), then herbivores (level 2), and then carnivores (level 3) and so on (see Fig. 1).

However, for high rates of recycling at the trophic level of zero, prokaryotic (heterotrophic bacteria and Archaea) detritivorus consumers are by far the most important group since the prokaryotic types can kill,

degrade, break down, digest, utilise and recycle any types of biomass at the higher trophic levels. In a detritus web, decomposers ultimately break down both plant and animal matter and the microbial processes of decomposition are described below.

[Figure 1: caption Ecological pyramids as visual representations of energy flow and/or biomass accumulation and/or populations at different trophic levels. The horizontal dimension represents the abundance or biomass at each level [13]. A biomass pyramid shows the total mass of the organisms that each trophic level occupies in an ecosystem. There must be higher amounts of biomass at the bottom of the pyramid to support the energy and biomass requirements of the higher trophic levels [14]. In general, growth rates, metabolic rates and recycling rates decrease with size (weight) of the animal or plant species, and/or size of the individual cell, if unicellular].

Microbial decomposition

The carbon and nutrients in dead organic matter are broken down by decomposition, which consists of three physicochemical processes: leaching, fragmentation and chemical or biochemical (enzymatic) alteration of the dead material. The released nutrients can then be used for microbial growth and metabolism, which returns carbon dioxide to the atmosphere where it can be (re-) used for photosynthesis. Approximately 90% of terrestrial net primary production goes directly from plant to decomposer [15].

Decomposition rates vary among ecosystems and depend upon the physicochemical environmental conditions (e.g. temperature, pH, redox), the type and quantity of nutrients (especially the main growth rate limiting nutrients), and the types of microbial species present in the microbial community of decomposers. It should be noted that in soils, decomposition rates are highest in wet or moist conditions with adequate levels of oxygen [15].

Since heterotrophic species are involved as decomposers, the breakdown rate by bacteria on dead matter is the most important feature for re-cycling elements. If the dead organic matter is polymeric (e.g. starch, cellulose, protein, pectin, chitin), then the best decomposers will be those with suitable extracellular hydrolytic digesting enzymes, since the digestion/solubilisation rate depends on depolymerisation activity external to the cells.

On the planet as a whole there are many different environments producing primary biomass (trees, forests, grasslands, marine macroalgae, algal blooms on lakes and at sea etc) so there is no shortage of primary biomass as fuel, fuel that ultimately gets broken down and transformed into new secondary biomass, by the various life-forms that eat the plants, or eat the plant-eaters. The end result of all the food chains is (ultimately) wet biomass formed via heterotrophic bacteria, which degrade, putrefy and otherwise break down or add to the organic matter; into more sludge and CO_2.

The slower growth rate of photosynthetic species compared to heterotrophic bacteria is thought to be due to the heavy energy cost of carrying out photosynthesis and low efficiency of the photosynthetic processes within the algal cells. Efficiency is the amount of solar energy used in photosynthesis as a percentage of the total available solar energy and only about half of the incoming sunlight is of the right frequencies to power photosynthesis. Only about 8% to 11% is absorbed by the plant and only 3% to 6% is actually used to drive the chemistry. But this depends on the kind of plant, the time of year, time of day, latitude, and other factors [16]. Heterotrophs, in contrast with photosynthetic autotrophs, obtain their energy from any previously formed organic biomass, rather than from sunlight and photosynthesis. There is no shortage of dead/living biomass to work with as fuel from almost anywhere on the planet. If it could be efficiently utilised as a fuel, it would represent an endless supply of energy, which on a per annum basis could be greater than that currently obtained through fossil fuel extractions.

Microbial fuel cells

The Microbial fuel cell has its origins some 100 years ago from its discovery and invention by Potter [1] in 1911. However, the fuel cells were regarded as being a laboratory curiosity at the time since the power levels produced were incredibly small (nW). Cohen [17] in 1931 created batch culture microbial half fuel cells that, when connected in series, were capable of producing over 35 volts, suggesting a half-cell equivalent of 75-80 MFC; but with only a current of around 2 milliamps (i.e. giving 70 mW of power). Six decades later, Habermann and Pommer (1991) [18] first reported that electrochemically active bacteria can produce natural shuttle molecules (hydrogen sulphide - H_2S) for electron transfer, instead of synthetic mediators – which had been used up to that time – and demonstrated the first MFC wastewater treatment system, based upon mixed sludge consortia. Up until this time, nearly all MFC were based on synthetic chemical mediators (e.g neutral red or methylene blue), and nearly all systems were based on batch culture. Bond and Lovley (2003) [19] demonstrated the anodophilic/exoelectric electron transport conduction properties of *Geobacter* species. Since this time, publications regarding experiments using pure or mixed species MFC have been growing exponentially, demonstrating increasing interest by researchers and groups from all around the world [20]. MFC are composed of two electrodes, with a gap or membrane between them. This allows charge to be transferred between the electrodes, the anode chamber, where the bacteria grow, and the cathode chamber, where the electrons react with oxygen in air to form water and/or hydrogen peroxide depending on conditions [21].

Anode (acetate): $C_2H_4O_2+2H_2O \rightarrow 2CO_2+8e^-+8H^+$

Anode (sucrose): $C_{12}H_{22}O_{11} + 13H_2O \rightarrow 12CO_2 + 48H^+ + 48e^-$

Cathode: $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$

$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$

The MFC is the only known technology that generates electricity from the breakdown of multiple substrates and sources of waste (i.e. fuel)[22]. The technology is versatile as it offers direct power but also feedstock treatment, nutrient recovery and re-cycling capabilities [23] and sensing [24] for real-time monitoring of processed substrates. With regard to improvements in power density and scale up, a large dichotomy of opinion has now surfaced regarding the best way to scale up MFC; large (> 25 ml up to 100's of litres) or small MFC (< 25ml). Small MFC can be typically from 10 ml down to microfluidic volumes (<1 ml), with highest power densities at the smallest scale. However, there is a theoretical minimum limit at the micro-fluidic scale due to the increasing energy requirements of moving liquids through micro-capillary sized tubes, which at some point require more power that can be produced by the micro-sized units. However, very small scale MFC may be used for purposes other than producing electrical energy, e.g. as sensing devices, where the advantage of having a fast response time outweighs the energy loss for function. Alternatively, very small MFC could be fed via capillary-surface action, not relying on gravity or fluidic pumping. In contrast, MFC at large scale have been proposed or built with thousands of litre volumes by increasing volume of all main "dimensions". The first large scale (660-gallon; 2500 L) microbial fuel cell system [25] was expected to produce 2 kilowatts of power - enough to power a single household. But in the end the maximal current that could be measured was 2A at 400mV (0.8 W) per unit (6 large units), which equals a total of 4.8W, a value that is only 0.24% of the predicted value of 2kW. In contrast, Ieropoulos *et al*. used small scale MFC to energise Ecobot-1 and continued with small scale systems for building EcoBots II and III,[26] and all further experimentation with EcoBots. Ieropoulos et al. [27] adopted the strategic view that miniaturisation and multiplication of small scale MFC was the best way to scale up for power density (of a stack) and also for creating small scale cascades [28] to produce a complete "treatment channel" so that BOD/COD could be reduced to the levels required for safe disposal. If a particular type of wastewater with high BOD was to be to be treated, the cascade could be increased in length (e.g. from 4 to 6 units per cascade) whilst for treating

relatively clean wastewater, the cascade could be reduced in length (e.g. from 4 to 2 units). One obvious advantage of small volume over large is that for small-MFC, scale-up is just a question of building multi-unit modules and multi-modular stacks, using components that can be mass produced. Providing each channel is isolated from all the others with regard to fluid-conductance of the liquid streams [29] the system will scale up proportionally with power to whatever size of stack is required in terms of either amount of power or amount of treatment required, providing the design does not allow for short-circuiting by fluidic conductance of electrons across the channels, so that channels can be successfully wired in series to boost voltage. There are fluid conductance problems when multiple electrodes are immersed in large volumes (large scale MFC or benthic systems), which suggests another advantage of remaining small; to avoid fluidic short-circuiting. Nevertheless, important improvements in power production have been reported recently for both large [30] as well as small [31] types of MFC, and both may have a future role to play in liquid waste treatments in the future. Other marked differences between large and small scale MFC units are set out in table 1.

Table 1: Comparisons of large volume (>100 ml) scale MFC and small volume (< 25 ml scale)

Feature compared	**Large scale (>100 ml)**	**Small scale (<25 ml)**
Dilution rate	ZERO for batch culture or LOW <0.1h^{-1} (i.e. MFC-Digester)	HIGH (>0.1h^{-1})
Perfusible electrodes (e.g. carbon veil) with high surface area of electrode at the micron scale)	NO: A tendency to use solid electrodes	YES
High surface area to volume ratio for the anode electrode and chamber	NO: A tendency towards being low	YES: and higher at decreasing volumes
Biofilm structure growing around the anode	High likelihood of being a Thick Diffusion limiting biofilm	Thin biofilm: Not diffusion limited at moderate to high flow rates
Electron transfer mechanisms	Mediators and direct conductance	Direct conductance at high flow rates Mixture of types at low flow rate
Carbon-energy fuels	Rich: with excess C/E (e.g. blackwater)	C/E limited (e.g. urine)
Feature compared	**Large scale (>100 ml)**	**Small scale (<25 ml)**
Need for digestion time for insoluble polymeric particles	Yes, zero or low flow rates and a long residence time (or HRT) required which large scale systems can meet	Yes, but more easily controlled in cascades by applying feedback of digestate containing hydrolase enzymes
Theoretical approach to biofilm formation and function	Locked into conventional biofilm paradigm (see figs 2 & 3)	Recognises exception to paradigm (see figs 2 & 3)
Portability of basic units	Low: Cannot be described as portable	High: From wearable (ultra-small) to large stacks of transportable modules
Manufacturing costs	High per unit	Low per unit, module and stack
Market sector	Restricted to bespoke water industries	For households and individuals as well as bespoke industries
Models of economic expansion, mass production and world distribution	Technology will be available to particular markets (e.g. wastewater companies) but not individuals or public at large since units too large or expensive for most individuals	Technology will be available across wide markets including individuals, households, farms, villages as well as large or small companies

The work by Ledezma et al.,[32] showed that the anodic biofilm specific growth rate can be both determined and controlled by changing substrate supply rate as found with other experimental loose matrix perfusion systems. This is an important finding, suggesting that for thin biofilms at moderate flow, diffusion limitation does not strongly feature and that nearly all current theoretical models of MFC are fine for thick film biofilms but do not particularly apply for thin perfusion films. For small scale MFC the rate-limiting step is the supply rate of growth limiting substrate by hydrodynamic flow and perfusate transport through the perfusion matrix. Feeding with nutrient-limiting conditions at a critical flow rate (50.8 mL h^{-1}) resulted in the first experimental determination of maximum specific biofilm growth rate, μ_{max} of 0.82 h^{-1} for *Shewanella* species as attached cells in biofilm mode [32]. This is considerably higher than those predicted or assumed via conventional modelling approaches. It is also shown that, under carbon-energy limiting conditions there is a strong direct relationship between growth rate and electrical power output, with μ_{max} coinciding with maximum electrical power production. Moreover, dynamic steady states of growth were apparent from 0.1h^{-1} up to 0.82h^{-1}. The data are incompatible with the conventional biofilm paradigm (see figure 2).

Biofilms

Helmstetter and Cummings (1963) [33] first demonstrated that populations of bacteria could be reliably produced when grown as a biofilm deposited on to a microporous membrane and perfused from the obverse side with sterile nutrient medium. New cell production reached a constant rate with time (quasi-steady state) suggesting that the attached biofilm layer was firmly attached to the membrane and could be described as a "mother layer" for all later progeny from the processes of cell division. The new cells detached and were described as "daughter cells" and production rate of these equalled their detachment-dissemination rate. A similar phenomenon was more closely observed by Gander and Gilbert (1997)[34], and again their work showed the possibility of precisely controlling the growth rate and physicochemical parameters in a similar way to that achieved in a chemostat, providing that the attachment matrix allowed for homogeneous perfusion of fresh nutrient medium to all cells [35]. A final elaboration of what has been termed an "in vitro matrix perfused biofilm" is a flat bed, described as a flatbed perfusion biofilm model [36]. All such models appear to show that under the right physicochemical conditions the biofilm remains fully viable as a fixed population of attached cells, with a long half-life of biofilm attachment (weeks), whilst daughter cells are splitting away constantly from the mother layer with a very short residence time equal to the growth rate of the system. However, careful analysis of the data from such perfusion systems suggest that steady states only last for a few weeks whilst the biofilm population density continues to slowly increase over days which suggests that the system is only quasi- or pseudo-steady state in behaviour, leading to the question of whether a close to true or ideal steady state biofilm bioreactor will ever be possible. However, the finding that perfusion type MFC can continue to function successfully over many years (>10 years in one experiment that is still ongoing) suggests that the biofilms that form are very stable with an ongoing fixed population number that does not build up into a thick, diffusion limited form. The special features that may account for the electrode biofilms to remain in close to perfect steady states for long periods of time are outlined in figure 2 and 3. Either naturally, or else depending on the hydrodynamic shear forces (set from the speed of the flow pump) the biofilm grows in steady state for as long as it is supplied with appropriate nutrients at a constant rate of flow. MFCs based on perfusable carbon veil electrodes have already demonstrated such dynamic steady states [32,37], even for complex mixed species, providing the system is carbon-energy limited and at moderate to high flow rates [38].

In contrast, for large scale volume MFC in batch culture or low flow mode, the C/E nutrients are usually supplied at high concentration, particularly at the start of the batch cycle. The biofilm that forms is thick containing large amounts of EPS (see figure 3). This is particularly true for electrode substrata that are impermeable to nutrient substrates. Daughter cells of the inner layer become entrapped as well as exogenous species, thickening the population; diffusion limitation builds up; chemical gradients form (figure 3) and the biofilm becomes very heterogeneous with starved inner layers and fast growing outer layers; the collective growth rate of the cells decrease because the inner layers become starved of nutrients; the system becomes sluggish and power output decreases. Moreover, dissemination of new cell growth is by erosion of clumps or aggregates - see figure 3a. For small-scale volume MFC this is not the case – see figure 3b. Here, dissemination is by random single cell release producing a fine suspension of cells in suspension. This is in contrast with the large volume system that produces large aggregate clumps of biofilm material appearing stochastically.

Spontaeous release of daughter cells may be a naturally occurring process for some species of bacteria (see later comments). However, the importance of hydrodynamic flow and fluidic shear forces cannot be ignored. Work by Boks et al., [39] studied the forces involved in bacterial adhesion to hydrophilic and hydrophobic surfaces. These workers concluded by showing that the hydrodynamic force to prevent adhesion (F_{prev}) is lower than the hydrodynamic force to stimulate detachment of a mature biofilm, (F_{det}) showing that the bond between a substratum surface and a bacterium becomes much stronger after initial adhesion. F_{det} is important to consider for biofilms. If the F_{det} for cells to substratum is higher than the F_{det} between mother cells and daughter cells after full septum formation, then liquid shear stress could be used to ensure a non-accumulative steady state by using high shear/high flow rates. The nearest description from a chemical engineering view is that the system behaves as a plug-flow reactor with biotransformations occurring by whole cell biocatalyst(s) attached to a stationary matrix within the system. The system is non-accumulative and can maintain dynamic steady state output for steady state conditions yet show changes in growth rate and electrical output that correspond to changes or perturbations in the physicochemical conditions. For a well-perfused biofilm in steady state (i.e. non accumulative), the specific biofilm growth rate (μ) is given by: μ = Rate of elution of daughter cells (h^{-1})/total biofilm population [32,37]. The relationships between growth rate, growth yield and concentration of growth limiting substrate (as previously proposed by Monod) can be applied to steady state perfused biofilm-electrodes. The Power output of the small scale MFC is the electron abstraction rate and this is strictly proportional to the metabolic rate (Q_{met}) of the microbial community around the anode electrode (the electrode-biofilm). In theoretical modelling, for a well-functioning small scale MFC at moderate to high flow rate, all terms for diffusion (such as Fick's law) are redundant. All mainstream models of MFC assume that the biofilm is strongly diffusion limiting, requiring complex models to make predictive statements about its kinetic features. Providing the fuel nutrient supply is carbon-energy limited, the Exocellular Polymeric Substance (EPS) production will be minimised. In addition the metabolic rate of the cells has also been strongly associated with the growth rate (since anabolism and catabolism are closely linked in C/E limiting conditions). It has been shown [40] that in continuous flow MFC differences in external resistance affect cellular electron transfer rates on a per cell basis as well as relate to the overall biofilm development in *Shewanella oneidensis* strain MR-1. When a low external resistance (100 Ω) was used, estimates of current per cell reached a maximum of 204 fA/cell, while when a higher (1 MΩ) resistance was used, only 75 fA/cell was produced. The 1 MΩ anode biomass consistently developed into a mature thick biofilm with tower morphology greater than 50 µm thick, whilst only a thin biofilm (<5 µm thick) was observed with the more powerful output with the 100 Ω anode. Likewise, for *G. sulfurreducens* biofilm [41], which reached the

highest electrochemical activity with a biofilm thickness of less than 20 µm, whilst with increasing thickness, the electrochemical activity decreased until the biofilm growth ceased at a thickness of ~45 µm. The electrochemical analysis and the metabolic spatial variability showed that (in batch culture development) a great many inactive cells accumulated resulting in high diffusion resistance. The authors concluded that although the *G. sulfurreducens* can always form thick biofilms, its highest electrochemical activity was reached at a much thinner thickness, suggesting that the inner layer(s) of live-cell mass, rather than the biofilm thickness per se, is responsible for the high current generation. Moreover, fast accumulation of inactive cells caused lower current generation in the thick biofilm. More recently, the same phenomena (high external resistance load, thick biofilm and low power output versus low resistance load, thin biofilm and high power output) was observed by Pasternak et al.,[42] but using more diverse mixed culture MFC.

The biofilm structure within an MFC depends upon many things resulting in either a thick (diffusion limiting) or thin (monolayer, bilayer or very thin multi-layer) perfusible structure. Whereas large scale, rich carbon-energy source biofilms at low or zero flow rate obey the current "thick" biofilm paradigm (see figure 2) this appears not to be the case with highly perfusible electrodes. [See Box 1: Below]

Box 1: Biofilm paradigms, stages from formation to dissipation

THICK biofilm: Conventional paradigm

- The initial (reversible) and later irreversible attachment processes are well described
- There is growth of attached cells and further attachment of cells from suspension
- Biofilm thickens to mature quasi-steady state, but biofilm is stratified with inner and outer layers with different growth rates because of diffusion, made worse when cells are fed on rich sources of C/E when they produce EPS.
- Inner layers decay due to lack of substrates
- Depending on hydrostatic or mechanical shear forces, erosion and internal decay weaken parts of the biofilm which break away as clumps or aggregates. The dissemination rates are stochastic.

THIN biofilm: Perfusion matrix paradigm

- The initial (reversible) and later irreversible attachment processes are well described
- There is both growth of attached cells and further attachment of planktonic cells from suspension
- When all attachment sites on the substratum are saturated by adherent cells the biofilm population thereafter remains more or less fixed in population
- All new progeny are naturally detached (needing little hydrodynamic shear) or more forcibly detached by high mechanical or hydrodynamic shear.
- All that is required is that the attachment forces between the substratum and mother layer are much stronger than the bonds that hold cells together following cell division. All newly grown cells detach from the mother layer at a rate that matches the rate of growth.
- Only at exceptionally high shear rates is the mother layer removed.
- Biofilms mature to quasi-steady state or possibly real steady state but remain active and fully functioning and very stable.
- New exogenous species cannot get to the mother layer due to exfoliation of daughter cells.

The Biofilm matrix for thick biofilm includes live and dead cells plus extracellular fluid, plus EPS. The turnover rate for the matrix fluid is relatively slow, due to diffusion. In contrast, the biofilm matrix for the thin biofilm includes living attached cells, the carbon veil substratum, liquid voids and channels. The voids and channels contain fluid but with very rapid turnover since it is perfused rather than diffused.

Studies on the control of biofilm formation and cell detachment by *Shewanella oneidensis* biofilms[43] have identified a set of genes responsible for both formation and detachment of biofilms and that cyclic di-GMP was

a key intracellular regulator for controlling biofilm stability, by shifting the state of a cell between attachment and detachment in a concentration-dependent manner. Such switching involves sophisticated signalling and tight regulation, with gene function and expression producing orchestrated protein interactions, many in response to the signalling molecule, cyclic-di-GMP. More recently, workers [44] have shown that three important genes, *BpfA*, (a biofilm-promoting protein), *BpfG* (a periplasmic transglutaminase-like cysteine proteinase) and *BpfD* (a bifunctional diguanylate cyclase/phosphodiesterase LapD-like protein), and together these regulate biofilm formation and detachment in *Shewanella oneidensis*. The genes and their expressed gene products were very similar to three homologous genes (the LapA-LapB-LapD network) found in the phylogenetically related *Pseudomonas fluorescens* [45]. A critical 1:1 ratio of BpfG and BpfD allows BpfA localization on the outer membrane, and biofilm forms. However, cyclic-di-GMP hydrolysation may allow disassociation from BpfD leading to BpfG release and activation of its proteinase activity such that BpfA is subsequently digested and the biofilm disperses. However, all the above *Shewanella* studies were conducted using plastic coated multiwell plates rather than on carbon matrix-flow substrata, nor were biofilms formed (or dispersed) from a polarised anodic electrode (MFC).

Power-producing bacteria

Electroactive bacteria transfer electrons to the anodes either directly or via self-produced mediators. In the direct mechanism [46,47], electron transfer occurs via membrane-associated c-cytochrome or through conductive pili or appendages. In mediated mechanisms, electron transfer between the bacterial cell and the anode surface occurs through artificial or self-produced soluble redox compounds such as flavins or pyocyanin [47]. *Shewanella* sp. appears to have both mechanisms; outer-membrane cytochromes and conductive pili as well as soluble mediators such as flavins, whilst *Pseudomonas sp.* appear to be entirely dependent on production of soluble mediators that shuttle electrons from the bacteria through the biofilm to the anode [48].

The ability to produce electrical power in MFC in the presence of artificial or natural redox mediator compounds is thought to be a fairly common property of most types of living cells, including bacterial heterotrophs. However, power-producing bacterial species that have evolved specific exoelectrogenic activity without requiring exogenous mediators are more infrequent. A published list [49] includes: *Shewanella* species, *Geobacter* species, *Rhodoferax ferrireducens*, *Rhodopseudomonas palustris*, *Ochrobactrum anthropi*, *Desulfovibrio desulfuricans*, *Desulfuromonas acetoxidans*, *Acidiphilium sp*. *Thermincola sp*. and *Cupriavidus basilensis*. Mixed bacteria usually produce higher power densities in MFCs than pure bacterial strains, but no single species can be directly compared with another, unless all environmental parameters are identical; yet identical parameters means that some species may be close to their optimum, whilst others would be sub-optimum.

Photobioreactors (PBRs):

A very wide range of types or designs of photobioreactors have been built for growing microalgae at large volume scale. A critical overview by Huang *et al.,* [50] of the key parameters that most influence the performance of PBRs, shows that both light intensity and spectral composition can most influence algal growth when light levels are growth rate limiting. However, mixing and mass transfer characteristics, temperature, pH, and also the capital and operating costs were all considered important. The lifespan and the costs of cleaning and temperature control are also emphasized for commercial exploitation, and four types of PBRs—tubular, plastic bag, column airlift, and flat-panel airlift reactors are recommended for large-scale operations [51]. Carbon dioxide and the laws governing algal growth when CO_2 levels are growth-rate-limiting, as well as laws governing algal growth rate and temperature are well established [52]. Algal species suitable for tropical climates may not suit temperate or cold regions of the

planet. In addition, essential micro-nutrients and tolerance of salt (i.e. freshwater or marine) are also important considerations.

More recently the value of immobilisation of algal cells has begun to be realised, in particular in a design or type of reactor known as a porous substrate bioreactor (PSBR) [53], also known as the twin-layer system [54] considered to be a new principle to separate the algae from a nutrient solution by means of a porous reactor surface on which the microalgae are trapped in biofilms. This procedure reduces the amount of liquid needed for operation by a factor of up to one hundred compared to conventional PBR technology, which cultivates algae solely by planktonic suspensions. As such, the PSBR procedure significantly reduces the energy needed while increasing the portfolio of algae that can be cultivated. It also allows for much higher growth rates and purity levels than found anywhere in nature.

Photoautotrophic cultivation of microalgae in suspensions is usually associated with a relatively low biomass content in the suspension of about 0.1–0.5% dry matter requiring high volumes of water. Moreover, the relatively low surface-to-volume ratio at high volume generates long path lengths for essential gases (CO_2 and O_2) and nutrients to diffuse into or out of the cell. The diffusion processes are thus too slow to support mass demands for efficient biomass growth, so mass transfer has to be driven actively, mostly by energy intensive mixing of liquid and/or bubble aeration adding to cost. Processing the algal biomass at low cell density is also problematic since it needs separating from a large volume of water using centrifugation or flocculation which are either energy- or cost-intensive procedures.

Generally, biofilm-based PBRs can be divided into submerged systems and porous substrate photobioreactors (PSBRs) [54]. In submerged-biofilm PBRs, the biofilms are usually immobilized/attached on to impermeable substrata either constantly, or periodically submerged, whilst in PSBR, the biofilm is attached to a microporous material, confined to one side of the substratum, separating the biofilm from the flow of medium [54–56,].

Biofilm-based PBRs have been used for production of microalgal biomass, production of microalgal-derived products (e.g. lipids, pigments, food), wastewater treatment and CO_2 sequestration [57,58]. All of these applications rely on rapid microalgal growth (i.e. high productivity), requiring high fixation rates of inorganic carbon. A comparison (by review) of algal raceway ponds, closed planktonic PBRs, Biofilm PBRs [conventional] and Biofilm PSBR [59–61] showed the PSBR to have the highest maximal biomass density (g dry weight L^{-1}) and the lowest consumption of water (i.e. volume to produce 1 kg of dry matter biomass). For example, such bioreactors have a surface productivity of 2 g m^{-2} day^{-1} and a flow rate of 6 L h^{-1} per meter of lighted photo module [62,63,]. Moreover, approximately 1 kWh is required for pumping to produce 1 kg of algal dry mass. In biofilms, the homogenous mixing of the biomass and the culture medium does not occur and mass transfer through dense biofilms relies largely on diffusion. It is well known that compared to advection (liquid flow), mass transfer through diffusion is much slower above the micrometer scale [64–66]as slow as it is for thick bacterial biofilms.

Integrating MFC with PSBR to produce PMFC

Photobiological systems based on microalgae or other photosynthetic microbes at the anode

There are a number of interesting combinations of MFC with photoelectric, photobioelectric, or simply photosynthetic systems [67–70]. Photobiological systems at the anode have been proposed based on cyanobacteria including *Synechocystis PCC-6803)* [71] and *Anabaena variabilis* [52] and other photosynthetic microbes (e.g. *Spirulina platensis* [72,73] and *Rhodospirillum rubrum* [74] and *Rhodobacter sphaeroides*[75]. Generally speaking, the use of an oxygenic species at the anode results in low

performance due to the high redox produced by oxygen at the anode, in contrast with anoxygenic (anaerobic) species which appear to show more promise. Photosynthesis close to the anode is also a property of "Plant Microbial Fuel Cells". Living plants are known to release a wide range of organic matters through their roots into the rhizosphere via photosynthesis [76–78]. The exudates contain water-soluble, water-insoluble and volatile compounds including a wide range of organic compounds that can be utilised by soil microbes and stimulate growth of the rhizospheric microorganisms [79] representing a large chemical energy flow into the soil matrix [80]. Plant species belong to one of three main types according to which of three pathways (C3, C4 and CAM) are used for photosynthesis and in general C4 plants have the highest photosynthesis efficiency for converting solar energy into bioelectricity [81] although some C3 plant species may exhibit higher efficiency than C4 plants [82]. Examples of named species includes *Oryza sativa* (Asian rice) or *Oryza glaberrima* (African rice) [83–85], *G. maxima*[86], *L. minuta* and *V. duckweed* [87,88,] and others, including ornamental plants [89]. However, the use of plants as sources of root exudate to the anodic microflora is an indirect process relying on soil diffusion to transport the nutrient components of the exudate to the anodic biofilms and this process is likely to be a relatively slow rate-limiting step compared with advective transport, or indeed the potential speed and growth rate of the microbes colonising the anodic electrodes.

Photobiological systems based on microalgae or other photosynthetic microbes at the cathode

PMFC that work with oxygenic cyanobacteria or microalgae in the cathode can improve the performance of the MFC by as much as 20% [90], probably due to oxygen being generated around the electrode within the chamber, but the system is also advantageous for sequestration of CO_2 in the presence of sunlight. This application helps in the wastewater treatment (through anodic oxidation and cathodic reduction) and sequestration of CO_2 (through cathodic reduction into cell biomass). Newly produced biomass in the cathode half-cell is supplemented by the cationic species (Na^+, K^+, NH_4^+) that are recycled from the anode to the cathode thanks to the proton/cation flow. New biomass grown in the cathodic photoreactor can then be used as a feedstock for the microbial anode in the same system [91]. It has been shown previously that algae powders (obtained by centrifugation or filtering, dried, ground into powder, and/or treated by acid-thermal processes) are suitable for bioelectricity production and are easily digested by the anodic MFCs [92]. An alternative is to use a flow-through system whereby the MFCs are continuously fed by algal biomass. Walter et al.[93] used a photo-chemostat to produce continuous culture of *Synechococcus leopoliensis* which continuously supplied a pre-digester initiating the digestion of the phototrophs and producing a fuel devoid of oxygen, and finally the outputs were fed continuously to a cascade of 9 MFCs, hydraulically and electrically independent. An earlier integrated photo-bioelectrochemical system for contaminants removal and bioenergy production has been reported [94] as well as an MFC with an algae-assisted cathode [95].

What MFC/PMFC can do in terms of treatment, recycling and power

The range of useful applications where MFC/PMFC could be utilised to benefit humanity is considerably wide (see figure 4). As such, MFC/PMFC processes and systems have been described as being a "platform technology" with uses and applications across a wide range of economic and industrial sectors (see table 2). An ideal PMFC would balance the elements by matching the digestion/utilisation rates of the detritivores with the primary biomass production rate of the microalgal cathodic side. It is unknown to what extent such a recycling machine could be adapted to recycle in the wider context of actual physical extraction of key bespoke elements such as P, S, and N. However, all machines should be able to utilise CO_2 and produce oxygen, water, biomass and energy.

Their useful properties may include the following:

1. Utilisation capability: What kind of materials can work as fuels:

From the literature it is obvious that MFC (whatever scale employed) are capable of utilising a very wide range of substrates, from monomers such as acetate,

lactate, glucose, to a wide range of carboxylic and hydroxyl-carboxylic acids, monosaccharides, disaccharides, polysaccharides (including cellulose, chitin, pectin), alcohols and aldehydes, amino acids, di-peptides, tri-peptides, peptides, proteins, phospholipids, amines, pentose, ribose, deoxyribose, nucleotides and nucleosides, RNA and DNA). It is generally thought that the best inocula to use for experiments are obtained from MFC already adapted to grow on a particular target substrate by species enrichment. Of particular interest is the ability of MFC to utilise methane [96] and other volatile compounds.

Some of the most difficult waste streams to treat are complex mixes of different substrate classes, such as sewage sludge, urine[97,98], brewery waste [99] and wastes that are polluted by heavy metals, examples including landfill leachate [100] but MFC appear to work in a satisfactory manner when supplied with almost anything organic, including petroleum hydrocarbons [101] and would probably utilise some of the many types of plastic materials, providing these were supplied in suitable form (e.g. as microplastic spheres).

2. Special products:

Biomass can be converted into liquid "pre-fuel" bulk chemicals (acetate, propionate, and *n*-butyrate) by fermentation using the carboxylate platform [102–104]. The carboxylate platform operates under non-sterile conditions and uses a mixed community of anaerobic microorganisms to convert lignocellulosic materials into the carboxylic acids. These pre-fuels are converted into real fuels by conventional chemical or enzymatic derivatisation (e.g. methylation or esterification) which can transform the carboxylic acids into alcohols, jet fuel, and gasoline. These features allow the platform to be flexible in terms of the variety of feedstocks it can accommodate, and cost-effective in that it does not necessarily require any additional chemicals or exogenous enzymes to carry out conversions.

It should be noted that in MFC, two important groups of microflora can be distinguished; the anodophile and the fermentative heterotrophs. Most electroactive species utilise short chain fatty acids, but not saccharides or polysaccharides. Most heterotrophs can hydrolyse the polymers and ferment the products of hydrolysis. In other words, the front-end reactions (hydrolysis of polymers and fermentation into short chain fatty acids) is common to both systems. The difference is that the fatty acids produced in the MFC are rapidly utilised *in situ* by the electron-generating organisms. However, by switching the electrical load of an MFC towards open circuit, it is possible to turn off the cells utilisation of the fatty acids, thereby allowing the anodic chamber to actually produce the products in question. The switching of MFC's within a stack should be periodic in order to maintain the ecological stability of the biofilm system.

Although mixed species consortia will be used in many types of MFC applications, it is likely that for producing particular bacterial products, a specific mixture of two species (heterotrophic species plus anodophile) would still allow for expression and harvesting of particular bacterial products such as exocellular enzymes (protease, nuclease, lipase, amylases, pectinases, chitinases, phospholipase) from natural or genetically engineered heterotrophs (e.g. *Escherichia coli*). Genetic constructs are available to make vaccine peptides, and many kinds of therapeutic or industrial proteins.

3. Cathodic products:

Under ideal conditions the cathode reactions should utilise oxygen, protons and electrons to produce water. However, on non-noble metal catalysts such as carbon electrodes, the reduction of oxygen in alkaline media usually follows a 2-electron mechanism (peroxide pathway) and hydrogen peroxide degradation into OH^- [105] resulting in local pH increase in the cathode and production of caustic agents.

Methods to harvest cathodic liquids have been previously discussed. Kim *et al*. [106] observed that anolyte loss varies with external resistance due to ionic flux driving the electro-osmotic transport of water and resulting in formation of liquid in a previously empty cathode. Of particular note is the finding [107] by Gajda et

al., that liquid (water) production rate increases in proportion to the power output of the MFC and that under sub-optimum electrode voltages, different catalytic events can be made to occur such as the production of OH^- and possibly hydroxyl radical and hydrogen peroxide that may have use as disinfecting agents [108]. Electro-osmotic production of purified catholyte can extract water from urine against osmotic pressure. Recovering valuable resources from urine such as energy and nutrients would help to transform energy intensive treatments to resource production [98,109]. Moreover, the use of transition metal ion catalysts particularly copper and iron coupled with low pH is an interesting way to produce an MFC-Fenton machine. An anodic Fenton system has been described [110] that was developed for both energy-saving and efficient treatment of organic pollutants by incorporating microbial fuel cell (MFC) into the process. The system was composed of an anodic Fenton reactor and a two-chamber air-cathode MFC. The power generated from the two-chamber MFC was used to drive the anodic Fenton process for Acid Orange 7 (AO7) degradation through accelerating the *in situ* generation of $Fe^{(2+)}$ from sacrificial iron. The high AO7 degradation rate clearly showed that the integrated MFC-anodic Fenton process could offer cost-effective and energy-saving electrochemical wastewater treatment. However, it is more usual to employ the cathodic compartment for breaking down recalcitrant compounds. For example, an in situ Fenton-enhanced cathodic MFC using a Fe-Fe_2O_3/carbon felt (CF) composite cathode has been described[111], that can both increase the power output of the MFC as well as offering a potential solution towards the degradation of many types of recalcitrant contaminants, including enhanced estrogen degradation [112].

Additional abilities when integrated with suitable algal species in PMFC [113]

4. Ability to utilise CO_2 and generate oxygen

5. Ability to "inoculate" larger volumes of photobioreactors to expand biomass production and rates of CO_2 utilisation and oxygen generation (i.e to act as a small repository of exponentially growing algae)

6. Algal biomass may be recycled via the anodic chamber

7. Algal biomass may consist of edible strains that can be used for human or animal nutrition

8. Algal biomass may consist of species that produce lipids and biodiesel.

9. Algal biomass may consist of species that produce useful chemical compounds [114,115]including biomass, biomass protein, polysaccharides, pigments, lutein, β-carotene, fucoxanthin, canthaxanthin, astaxanthin, phycocyanin and fatty acids including docosahexaenoic acid, eicosapentaenoic acid, arachidonic acid, and γ-linolenic acid.

10. Algal biomass can be used for carbon capture and storage: Algal biomass can be pyrolysed and buried

11. Ability to do all above whilst producing, rather than consuming electrical energy

12. System can be monitored and controlled *in situ* and fully automated or roboticised.

Future developments

In the near future it may be possible to develop and build machines consisting of both MFC and PMFC that could be described as "Energy Autonomous Biomass Transformers, or Recyclers, or Trans-cyclers" for relatively quick re-cycling of all of life's essential elements (CHONPS), using natural microbes (bacteria and microalgae) and utilising the power of autonomous robotic systems [116–119] and Artificial Intelligence [37,120–122,]to treat waste to make it clean whilst producing electricity, but also to produce safe fertiliser, algal biofuels or edible algae. The machine may treat a wide range of different types of waste; from urine to sludge and be used anywhere in the world where there is a supply of organic matter as fuel. As well as recycling

elements important for life it could be used to recover by extraction, water, phosphate, sulphur, oxygen or recover new biomass for carbon-capture and deep burial. All MFC/PMFC systems have the advantage of generating electricity and operating autonomously.

Conclusions

The prokaryotic bacteria and archaea exhibit an astonishing metabolic diversity, which far exceeds that of animals, plants, fungi and other higher organisms "put together". The prokaryotes literally keep our biological world turning by recycling all the mineral elements necessary for life support. For recycling purposes, the question is, which groups of bacteria and microalgae are the fastest growing, and how can we best exploit their superior growth rates.

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References:

1 M. C. Potter, *Proc. R. Soc. B Biol. Sci.*, 1911, **84**, 260–276.
2 J. Greenman and I. A. Ieropoulos, *J. Power Sources*, 2017, **356**, 365–370.
3 J. P. DeLong, J. G. Okie, M. E. Moses, R. M. Sibly and J. H. Brown, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 12941–5.
4 M. T. Weinstock, E. D. Hesek, C. M. Wilson and D. G. Gibson, *Nat. Methods*, 2016, **13**, 849–851.
5 M. Lürling, F. Eshetu, E. J. Faassen, S. Kosten and V. L. M. Huszar, *Freshw. Biol.*, 2013, **58**, 552–559.
6 R. M. Schuurmans, P. van Alphen, J. M. Schuurmans, H. C. P. Matthijs and K. J. Hellingwerf, *PLoS One*, 2015, **10**, e0139061.
7 J. Yu, M. Liberton, P. F. Cliften, R. D. Head, J. M. Jacobs, R. D. Smith, D. W. Koppenaal, J. J. Brand and H. B. Pakrasi, *Sci. Rep.*, 2015, **5**, 8132.
8 L. Silva, M. L. Calleja, T. M. Huete-Stauffer, S. Ivetic, M. I. Ansari, M. Viegas and X. A. G. Morán, *Front. Microbiol.*, 2019, **9**, 3244.
9 J. B. Cotner and B. A. Biddanda, *Ecosystems*, 2002, **5**, 105–121.
10 D. L. Kirchman, *Proc. Natl. Acad. Sci.*, 2008, **105**, 8487–8488.
11 D. L. Kirchman, *Ann. Rev. Mar. Sci.*, 2016, **8**, 285–309.
12 K. H. (Kenneth H. Mann, *Ecology of coastal waters : a systems approach*, University of California Press, 1982.
13 J. E. Duffy, B. J. Cardinale, K. E. France, P. B. McIntyre, E. Thébault and M. Loreau, *Ecol. Lett.*, 2007, **10**, 522–538.
14 N. G. Hairston, and N. G. Hairston, *Am. Nat.*, 2002, **142**, 379–411.
15 F. S. Chapin, P. A. Matson and P. M. Vitousek, in *Principles of Terrestrial Ecosystem Ecology*, Springer New York, New York, NY, 2011, pp. 3–22.
16 D. Y. Goswami, F. Kreith and F. Kreith, *Handbook of Energy Efficiency and Renewable Energy*, CRC Press, 2007, vol. 20070849.
17 B. Cohen, *J. Bacteriol.*, 1931, **21**, 18–19.
18 W. Habermann and E. Pommer, *Appl. Microbiol. Biotechnol.*, 1991, **35**, 128–133.
19 D. Bond and D. Lovley, *Appl. Environ. Microbiol.*, 2003, **69**, 1548–1555.
20 C. Santoro, C. Arbizzani, B. Erable and I. Ieropoulos, *J. Power Sources*, 2017, **356**, 225–244.
21 B. E. Logan, B. Hamelers, R. Rozendal, U. Schröder, J. Keller, S. Freguia, P. Aelterman, W. Verstraete and K. Rabaey, *Environ. Sci. Technol.*, 2006, **40**, 5181–5192.
22 P. Pandey, V. N. Shinde, R. L. Deopurkar, S. P. Kale, S. A. Patil and D. Pant, *Appl. Energy*, 2016, **168**, 706–723.
23 P. Kuntke, K. M. Smiech, H. Bruning, G. Zeeman, M. Saakes, T. H. J. a Sleutels, H. V. M. Hamelers and C. J. N. Buisman, *Water Res.*, 2012, **46**, 2627–36.
24 Y. Jiang, X. Yang, P. Liang, P. Liu and X. Huang, *Renew. Sustain. Energy Rev.*, 2018, **81**, 292–305.
25 J. Keller and K. Rabaey, in *Microbial Fuel Cells First International Symposium*, 2008, p. 13.
26 I. Ieropoulos, C. Melhuish and J. Greenman, in *Advances in Artificial Life*, 2003.
27 I. Ieropoulos, J. Greenman and C. Melhuish, *Int. J. Energy Res.*, 2008, **32**, 1228–1240.
28 J. Winfield, I. Ieropoulos and J. Greenman, *Bioresour. Technol.*, 2012, **110**, 245–50.
29 I. A. Ieropoulos, J. Winfield, J. Greenman and C. Melhuish, in *ECS Transactions*, The Electrochemical Society, 2010, vol. 28, pp. 1–9.
30 B. Min and B. E. Logan, *Environ. Sci. Technol.*, 2004, **38**, 5809–5814.
31 I. Gajda, A. Stinchcombe, I. Merino-Jimenez, G. Pasternak, D. Sanchez-Herranz, J. Greenman and I. A. Ieropoulos, *Front. Energy Res.*, 2018, **6**, 84.
32 P. Ledezma, J. Greenman and I. Ieropoulos, *Bioresour. Technol.*, 2012, **118**, 615–8.
33 C. E. HELMSTETTER and D. J. CUMMINGS, *Proc. Natl. Acad. Sci. U. S. A.*, 1963, **50**, 767–74.
34 S. Gander and P. Gilbert, *J. Antimicrob. Chemother.*, 1997, **40**, 329–334.
35 P. Gilbert, D. G. Allison, D. J. Evans, P. S. Handley and M. R. Brown, *Appl. Environ. Microbiol.*, 1989, **55**, 1308–11.
36 R. M. S. Thorn and J. Greenman, *J. Appl. Microbiol.*, 2009, **107**, 2070–2079.
37 J. Greenman, I. Ieropoulos and C. Melhuish, *Biological Computing Using Perfusion Anodophile Biofilm Electrodes (PABE)*, vol. 4.
38 J. You, J. Greenman, I. Ieropoulos, J. You, J. Greenman and I. Ieropoulos, *Energies*, 2018, **11**, 2377.
39 N. P. Boks, W. Norde, H. C. van der Mei and H. J. Busscher, *Microbiology*, 2008, **154**, 3122–3133.
40 J. S. McLean, G. Wanger, Y. A. Gorby, M. Wainstein, J. McQuaid, S. ichi Ishii, O. Bretschger, H. Beyenal and K. H. Nealson, *Environ. Sci. Technol.*, 2010, **44**, 2721–2727.

41 D. Sun, J. Chen, H. Huang, W. Liu, Y. Ye and S. Cheng, *Int. J. Hydrogen Energy*, 2016, **41**, 16523–16528.
42 G. Pasternak, J. Greenman and I. Ieropoulos, *J. Power Sources*, 2018, **400**, 392–401.
43 K. M. Thormann, S. Duttler, R. M. Saville, M. Hyodo, S. Shukla, Y. Hayakawa and A. M. Spormann, *J. Bacteriol.*, 2006, **188**, 2681–91.
44 G. Zhou, J. Yuan and H. Gao, *Front. Microbiol.*, 2015, **6**, 790.
45 L. Wu, X. Liu, M. W. Fields, D. K. Thompson, C. E. Bagwell, J. M. Tiedje, T. C. Hazen and J. Zhou, *ISME J.*, 2008, **2**, 642–655.
46 T. Mehta, M. V. Coppi, S. E. Childers and D. R. Lovley, *Appl. Environ. Microbiol.*, 2005, **71**, 8634–8641.
47 G. Reguera, K. D. McCarthy, T. Mehta, J. S. Nicoll, M. T. Tuominen and D. R. Lovley, *Nature*, 2005, **435**, 1098–101.
48 E. Marsili, D. B. Baron, I. D. Shikhare, D. Coursolle, J. A. Gralnick and D. R. Bond, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 3968–73.
49 M. Schechter, A. Schechter, S. Rozenfeld, E. Efrat and R. Cahan, in *Technology and Application of Microbial Fuel Cells*, InTech, 2014.
50 Q. Huang, F. Jiang, L. Wang and C. Yang, *Engineering*, 2017, **3**, 318–329.
51 S. P. Singh and P. Singh, *Renew. Sustain. Energy Rev.*, 2014, 38, 172–179.
52 T. E. Murphy and H. Berberoglu, *Biotechnol. Prog.*, 2014, **30**, 348–359.
53 J. Shi, B. Podola and M. Melkonian, *J. Appl. Phycol.*, 2007, **19**, 417–423.
54 B. Podola, T. Li and M. Melkonian, *Trends Biotechnol.*, 2017, **35**, 121–132.
55 T. Naumann, Z. Çebi, B. Podola and M. Melkonian, *J. Appl. Phycol.*, 2013, **25**, 1413–1420.
56 T. Naumann, Z. Çebi, B. Podola and M. Melkonian, *J. Appl. Phycol.*, 2013, **25**, 1619–1619.
57 L. Gouveia, A. E. Marques, J. M. Sousa, P. Moura and N. M. Bandarra, *Food Sci. Technol. Bull. Funct. Foods*, 2010, **7**, 21–37.
58 M. I. Khan, J. H. Shin and J. D. Kim, *Microb. Cell Fact.*, 2018, **17**, 36.
59 T. Li, M. Strous and M. Melkonian, *FEMS Microbiol. Lett.*, 2017, **364**.
60 G. Olivieri, P. Salatino and A. Marzocchella, *J. Chem. Technol. Biotechnol.*, 2014, **89**, 178–195.
61 M. Gross, D. Jarboe and Z. Wen, *Appl. Microbiol. Biotechnol.*, 2015, **99**, 5781–5789.
62 L. Katarzyna, G. Sai and O. Avijeet Singh, *Renew. Sustain. Energy Rev.*, 2015, 42, 1418–1427.
63 M. Kesaano, R. D. Gardner, K. Moll, E. Lauchnor, R. Gerlach, B. M. Peyton and R. C. Sims, *Bioresour. Technol.*, 2015, **180**, 7–15.
64 S. P. Cuellar-Bermudez, G. S. Aleman-Nava, R. Chandra, J. S. Garcia-Perez, J. R. Contreras-Angulo, G. Markou, K. Muylaert, B. E. Rittmann and R. Parra-Saldivar, *Algal Res.*, 2017, **24**, 438–449.
65 D. Hoh, S. Watson and E. Kan, *Chem. Eng. J.*, 2016, **287**, 466–473.
66 H. Siegrist and W. Gujer, *Water Res.*, 1985, **19**, 1369–1378.
67 M. Rosenbaum, Z. He and L. T. Angenent, *Curr. Opin. Biotechnol.*, 2010, **21**, 259–64.
68 A. Cereda, A. Hitchcock, M. D. Symes, L. Cronin, T. S. Bibby and A. K. Jones, *PLoS One*, 2014, **9**, e91484.
69 Y. Zou, J. Pisciotta, R. B. Billmyre and I. V Baskakov, *Biotechnol. Bioeng.*, 2009, **104**, 939–46.
70 S. K. Liehr, M. T. Suidan and J. W. Eheart, *J. Environ. Eng.*, 1989, **115**, 320–335.
71 P. Bombelli, M. Zarrouati, R. J. Thorne, K. Schneider, S. J. L. Rowden, A. Ali, K. Yunus, P. J. Cameron, A. C. Fisher, D. Ian Wilson, C. J. Howe and A. J. McCormick, *Phys. Chem. Chem. Phys.*, 2012, **14**, 12221.
72 C.-C. Lin, C.-H. Wei, C.-I. Chen, C.-J. Shieh and Y.-C. Liu, *Bioresour. Technol.*, 2013, **135**, 640–643.
73 C.-C. Fu, T.-C. Hung, W.-T. Wu, T.-C. Wen and C.-H. Su, *Biochem. Eng. J.*, 2010, **52**, 175–180.
74 M. V Gomez, G. Mai, T. Greenwood and J. P. Mullins, *J. Emerg. Investig.*, 2014.
75 Y. K. Cho, T. J. Donohue, I. Tejedor, M. A. Anderson, K. D. McMahon and D. R. Noguera, *J. Appl. Microbiol.*, 2008, **104**, 640–650.
76 A. D. Rovira, *Plant Soil*, 1959, **11**, 53–64.
77 Y. Goto, N. Yoshida, Y. Umeyama, T. Yamada, R. Tero and A. Hiraishi, *Front. Bioeng. Biotechnol.*, 2015, **3**, 42.
78 R. Nitisoravut and R. Regmi, *Renew. Sustain. Energy Rev.*, 2017, **76**, 81–89.
79 E. Paterson, *Eur. J. Soil Sci.*, 2003, **54**, 741–750.
80 F. D. Dakora and D. A. Phillips, *Plant Soil*, 245, 201–213.
81 C. Wang, L. Guo, Y. Li and Z. Wang, *BMC Syst. Biol.*, 2012, **6**, S9.
82 M. Helder, D. P. B. T. B. Strik, H. V. M. Hamelers, A. J. Kuhn, C. Blok and C. J. N. Buisman, *Bioresour. Technol.*, 2010, **101**, 3541–3547.
83 N. Kaku, N. Yonezawa, Y. Kodama and K. Watanabe, *Appl. Microbiol. Biotechnol.*, 2008, **79**, 43–49.
84 M. A. Moqsud, J. Yoshitake, Q. S. Bushra, M. Hyodo, K. Omine and D. Strik, *Waste Manag.*, 2015, **36**, 63–69.
85 L. De Schamphelaire, L. Van den Bossche, H. S. Dang, M. Höfte, N. Boon, K. Rabaey and W. Verstraete, *Environ. Sci. Technol.*, 2008, **42**, 3053–3058.
86 D. P. B. T. B. Strik, H. V. M. Hamelers (Bert), J. F. H. Snel and C. J. N. Buisman, *Int. J. Energy Res.*, 2008, **32**, 870–876.
87 Y. Hubenova and M. Mitov, *Bioelectrochemistry*, 2012, **87**, 185–191.
88 Y. Hubenova and M. Mitov, *Bioelectrochemistry*, 2015, **106**, 226–231.
89 Y. M. Azri, I. Tou, M. Sadi and L. Benhabyles, *Int. J. Green Energy*, 2018, **15**, 254–263.
90 I. Gajda, J. Greenman, C. Melhuish and I. Ieropoulos, *Int. J. Hydrogen Energy*, 2013, **38**, 11559–11564.
91 I. Gajda, J. Greenman, C. Melhuish and I. Ieropoulos, *Biomass and Bioenergy*, 2015, **82**, 87–93.
92 S. B. Velasquez-Orta, T. P. Curtis and B. E. Logan, *Biotechnol. Bioeng.*, 2009, **103**, 1068–76.
93 X. A. Walter, J. Greenman, B. Taylor and I. A. Ieropoulos, *Algal Res.*, 2015, **11**, 103–107.
94 L. Xiao, E. B. Young, J. a Berges and Z. He, *Environ. Sci. Technol.*, 2012, **46**, 11459–66.
95 A. González del Campo, P. Cañizares, M. a. Rodrigo, F. J. Fernández and J. Lobato, *J. Power Sources*, 2013, **242**, 638–645.
96 M. J. McAnulty, V. G. Poosarla, K.-Y. Kim, R. Jasso-Chávez, B. E. Logan and T. K. Wood, *Nat. Commun.*, 2017, **8**, 15419.
97 I. Ieropoulos, J. Greenman and C. Melhuish, *Phys. Chem. Chem. Phys.*, 2012, **14**, 94–8.
98 I. Gajda, J. Greenman, C. Santoro, A. Serov, P. Atanassov, C. Melhuish and I. A. Ieropoulos, *J. Chem. Technol. Biotechnol.*, 2018.
99 L. Zhuang, Y. Yuan, Y. Wang and S. Zhou, *Bioresour. Technol.*, 2012, **123**, 406–412.
100 J. Greenman, A. Gálvez, L. Giusti and I. Ieropoulos,

Enzyme Microb. Technol., 2009, **44**, 112–119.
101 O. Adelaja, T. Keshavarz and G. Kyazze, *J. Hazard. Mater.*, 2015, **283**, 211–217.
102 E. B. Hollister, A. K. Forrest, H. H. Wilkinson, D. J. Ebbole, S. A. Malfatti, S. G. Tringe, M. T. Holtzapple and T. J. Gentry, *Appl. Microbiol. Biotechnol.*, 2010, **88**, 389–399.
103 M. T. Agler, B. A. Wrenn, S. H. Zinder and L. T. Angenent, *Trends Biotechnol.*, 2011, **29**, 70–8.
104 E. B. Hollister, A. K. Forrest, H. H. Wilkinson, D. J. Ebbole, S. G. Tringe, S. A. Malfatti, M. T. Holtzapple and T. J. Gentry, *PLoS One*, 2012, **7**, e39689.
105 K. Kinoshita, *Carbon Electrochemical and Physicochemical Properties*, John Wiley & Sons, Ltd,., 1988.
106 J. R. Kim, G. C. Premier, F. R. Hawkes, R. M. Dinsdale and A. J. Guwy, *J. Power Sources*, 2009, **187**, 393–399.
107 I. Gajda, J. Greenman, C. Melhuish, C. Santoro, B. Li, P. Cristiani and I. Ieropoulos, *Sustain. Energy Technol. Assessments*, 2014, **7**.
108 I. Gajda, J. Greenman, C. Melhuish and I. A. Ieropoulos, *Sci. Rep.*, 2016, **In Press**, 25571.
109 S. Lu, H. Li, G. Tan, F. Wen, M. T. Flynn and X. Zhu, *Chem. Eng. J.*, 2019, **373**, 1072–1080.
110 X.-W. Liu, X.-F. Sun, D.-B. Li, W.-W. Li, Y.-X. Huang, G.-P. Sheng and H.-Q. Yu, *Water Res.*, 2012, **46**, 4371–4378.
111 L. Zhuang, S. Zhou, Y. Li, T. Liu and D. Huang, *J. Power Sources*, 2010, **195**, 1379–1382.
112 N. Xu, Y. Zhang, H. Tao, S. Zhou and Y. Zeng, *Bioresour. Technol.*, 2013, **138**, 136–140.
113 M. Shukla and S. Kumar, *Renew. Sustain. Energy Rev.*, 2018, **82**, 402–414.
114 O. Pulz and W. Gross, *Appl. Microbiol. Biotechnol.*, 2004, **65**, 635–648.
115 S. P. Cuellar-Bermudez, I. Aguilar-Hernandez, D. L. Cardenas-Chavez, N. Ornelas-Soto, M. A. Romero-Ogawa and R. Parra-Saldivar, *Microb. Biotechnol.*, 2015, **8**, 190–209.
116 I. Ieropoulos, J. Greenman, C. Melhuish and I. Horsfield, *ALIFE*, 2010, 733–740.
117 G. Papaharalabos, J. Greenman, A. Stinchcombe, I. Horsfield, C. Melhuish and I. Ieropoulos, *J. Power Sources*, 2014, **272**, 34–38.
118 P. Ledezma, A. Stinchcombe, J. Greenman and I. Ieropoulos, *Phys. Chem. Chem. Phys.*, 2013, **15**, 2278–81.
119 I. A. Ieropoulos, J. Greenman, C. Melhuish and I. Horsfield, *ChemSusChem*, 2012, **5**, 1020–1026.
120 J. Greenman, I. A. Ieropoulos and C. Melhuish, Springer, New York, NY, 2011, pp. 239–290.
121 I. A. Ieropoulos, J. Greenman, C. Melhuish and I. Horsfield, in *Artificial Life Models in Hardware*, Springer London, London, 2009, pp. 185–211.
122 M.-A. I. Tsompanas, A. Adamatzky, G. C. Sirakoulis, J. Greenman and I. Ieropoulos, *PLoS One*, 2017, **12**, e0177528.

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Fig. 1.

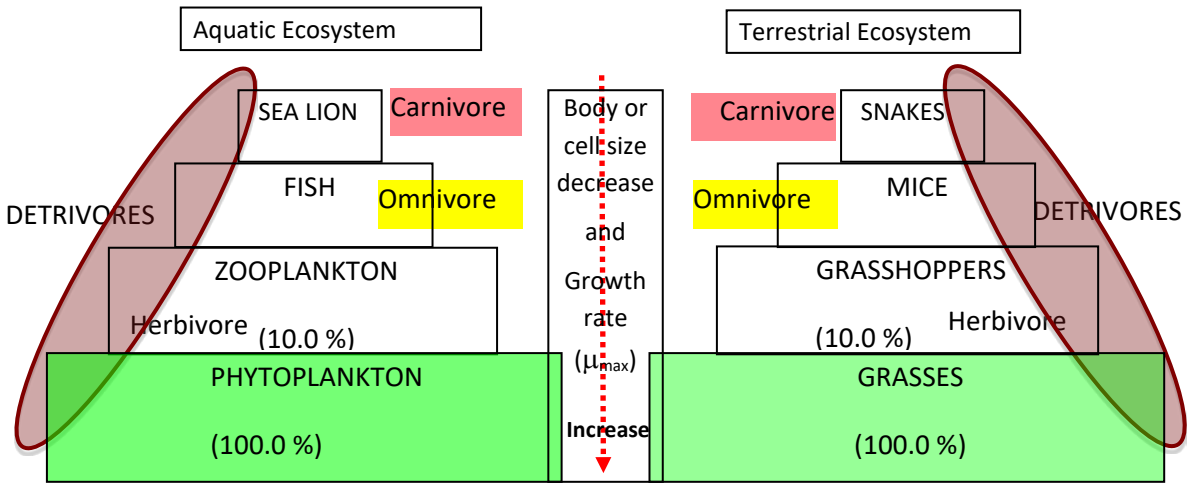

Figure 1: Ecological pyramids as visual representations of energy flow, biomass accumulation, and populations at different trophic levels. The horizontal dimension represents the abundance or biomass at each level [13]. A biomass pyramid shows the total mass of the organisms that each trophic level occupies in an ecosystem. There must be higher amounts of biomass at the bottom of the pyramid to support the energy and biomass requirements of the higher trophic levels [14]. In general, growth rates, metabolic rates and recycling rates decrease with size (weight) of the animal or plant species, and/or size of the individual cell, if unicellular.

Fig. 2.

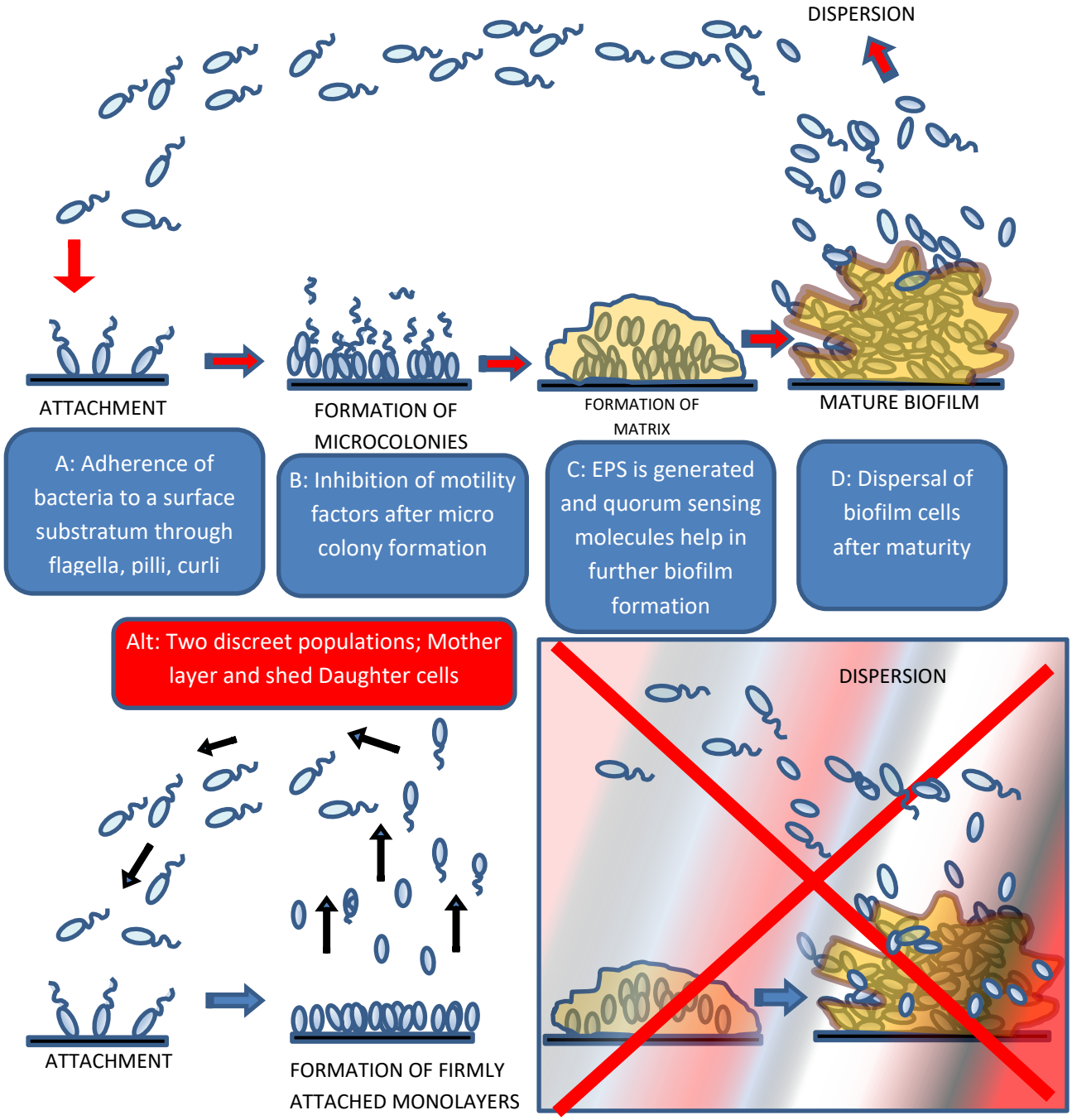

Figure 2: Biofilm paradigms: Attachment (A), formation of a mature biofilm (B and C) and dispersal of cells by detachment (D). The diagram below the blue boxes depicts an alternative view (Alt) and shows how this differs from the conventional view. However, if detachment can take place following growth of a monolayer or thin film layer, the behaviour of the system will be different to that of a conventional biofilm, particularly with regard to growth-diffusion effects of thick biofilms. These stages do not occur for “naturally” thin biofilms. For some species of bacteria the biofilm will be very stable with time with mother layers lasting for the whole lifetime of the system which may be many years.

Fig. 3.

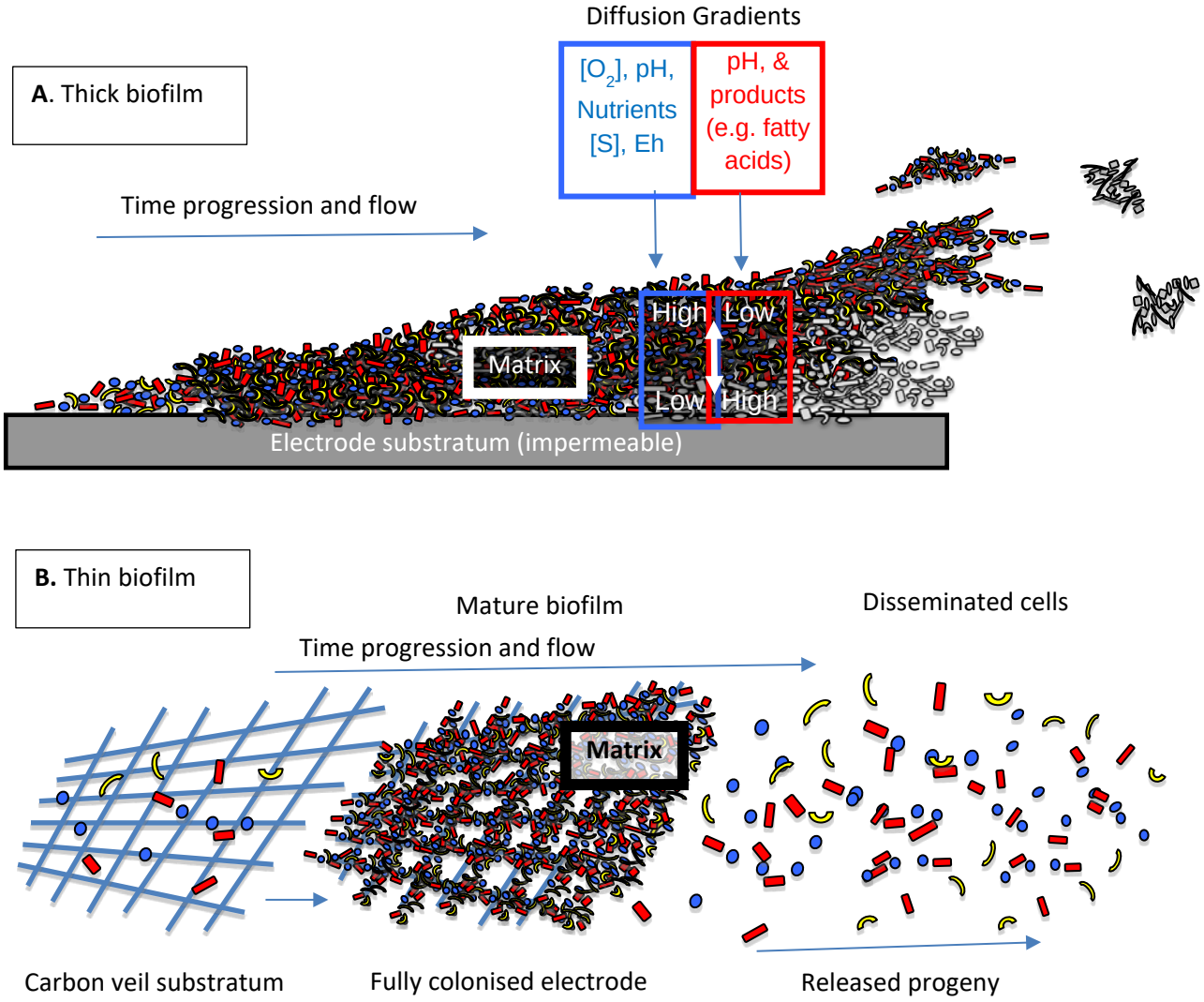

Figure 3. Illustrating the main differences between thick film and thin film biofilms: Although the phases of initial colonisation (weak and strong attachment followed by growth and extension) are the same, the way the biofilm processes proceed from this point onwards are very different. One type (A) forms diffusion limiting thick biofilm (only capable of quasi-steady state in a flow system), whilst the other (B) produces a thin perfusible biofilm consisting of permanently attached cells with new cell progeny (daughter cells) which, due to natural processes of detachment and/or hydrodynamic shear forces, wash away from the fixed and stable mother layer. In the case of the thick biofilm, the matrix does not include the impermeable electrode substratum, and as a result builds up a thick diffusion limiting biofilm with gradients and resultant heterogeneity of matrix. In the case of the thin biofilm, the matrix consists of liquid channels and voids in addition to cells and (if C/E limited) produces little in the way of cell EPS-material.

Fig 4.

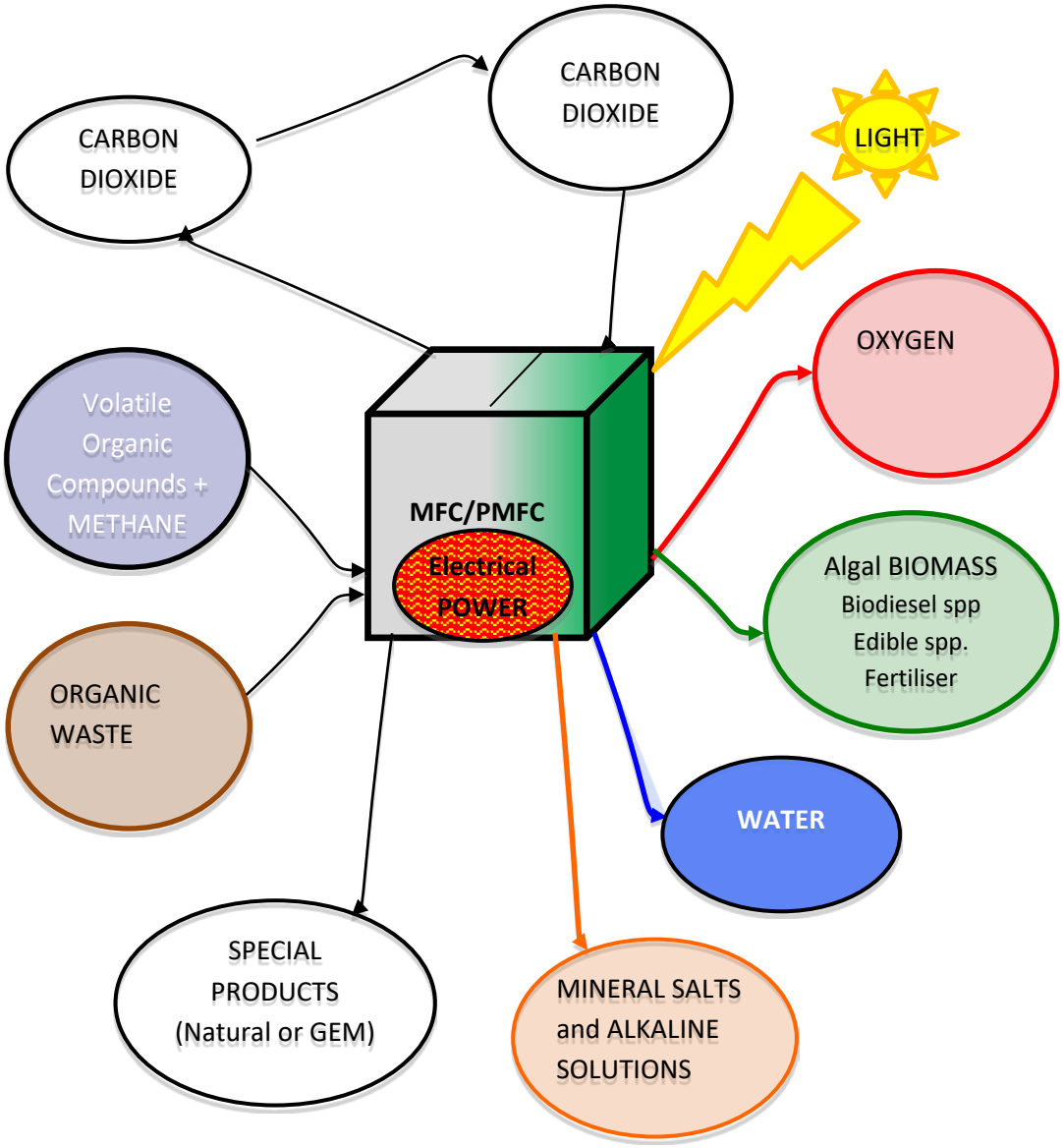

Figure 4: Potential capability of MFC/PMFC in terms of treatment, products, recycling and power

MFC treatment of organic waste: MFC capable of utilising a very wide range of substrates, including volatile organic compounds (including methane) and some types of plastics. MFC special products: MFC carboxylate platform, extracellular hydrolases, intracellular products from natural or genetically engineered heterotrophs to make vaccine peptides, and many other kinds of therapeutic or industrial proteins. MFC/PMFC synthesises water: from the cathode reactions. MFC/PMFC cathodic products: By fine tuning the electrode properties, different catalytic events can be made to occur such as the production of hydroxyl radical and hydrogen peroxide and therefore the use of solutions as disinfecting agents. PMFC ability to utilise CO_2: PMFC ability to generate oxygen using oxygenic microalgae: PMFC ability to generate other special algal products when integrated with suitable algal species PMFC ability to act as repository of active (exponentially growing) algae as inoculum for larger scale photoalgal bioreactors to expand biomass production as well as rates of CO_2 utilisation and oxygen generation. PMFC ability to produce algal biomass that may be recycled via the anodic chamber. PMFC algal biomass may consist of edible strains that can be used for human or animal nutrition, or species that produce lipids and biodiesel. PMFC algal biomass may be used for carbon capture and storage: e.g. biomass can be pyrolysed and buried: PMFC ability to produce electrical power: to do all above whilst producing, rather than consuming electrical energy. PMFC system that can be monitored and controlled *in situ* and fully automated or roboticised.

Table 2: Future examples of where the MFC platform may fit in as New Tech

SECTORS	PRIMARY	SECONDARY	TERTIARY	QUATERNARY
	Energy & resource recovery water re-cycling & mineral extraction	Light & heavy industries: chemicals, food medical/pharma, paper/pulp, biofuels, wastewater treatment, bioreactors	Manufacturing & marketing services, repair & maintenance Design, patents, insurance, financial	IT, Robotics, Electronics, A-Life, Artificial Intelligence
ROLE FOR MFC/PMFC	YES	SOME	INCREASINGLY	POSSIBLY
	e.g. green chemistry; bulk chem fine chem Energy and resource recovery Water recycling Carbon capture	e.g. biotechnologies: specific utilisation and/or specific production of: biomass, proteins, enzymes, polymers, algal food, etc. (including GEM products).	e.g. future need for MFC / PMFC "servicing" investment and insurance for those using the technology	e.g. bioelectronics Biohybrid devices, Living sensors EcoBots

Definitions:

'Platform Technology': One that can use the same fundamental system or base technology to drive a wide range of functions, applications or technologies across various sectors of the economy.
Primary sectors: Industries whose main function is to extract resources or make raw materials (e.g coal, oil, water, minerals, agricultural produce)
Secondary sectors: Industries that process resources/raw materials into manufactured goods and products [includes fermentation biotechnologies and green chemistry].
Tertiary sectors: Design, manufacturing, repair, maintenance, investment, insurance [i.e. service industries]
Quaternary: IT, Robotics, Electronics, A-Life, Artificial Intelligence