Electro-fermentation: How to drive fermentation using electrochemical systems

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Abstract

Electro-fermentation is a novel process that consists of electrochemically controlling microbial fermentative metabolisms with electrodes. The electrodes can act as electron sinks or sources that allow unbalanced fermentation. They can also modify the medium by changing the redox balance. Such an electrochemical control presents significant effects not only on microbial metabolism and biological regulations, but also on inter-species interactions and the selection of bacterial populations when using mixed microbial cultures. In this paper, we propose some basics and principles to better define the electro-fermentation concept within the field of bioelectrochemistry. We also explore the up-to-date strategies to put electro-fermentation into practice and propose hypothetical mechanisms that could explain the first electro-fermentation results reported in the literature.

Keywords: Bioelectrochemical systems; ORP; Interspecies electron transfer; Electromicrobiology; Electro-fermentation

From microbial fuel cells to electro-fermentation

Bioelectrochemical systems (BESs, see Glossary) correspond to a type of bioreactors in which both biological and electrochemical processes can occur to generate electricity, hydrogen or other products of interest. To differentiate the various types of BESs, usually, a new name is given according to the product or service that is provided [1]. Initially, research on BESs mainly focused on the production of electricity in **microbial fuel cells (MFCs)** [2-5]. Over the years, BESs have been used for many other applications, such as hydrogen production in **microbial electrolysis cells (MECs)** [6-7], chemical production from CO_2 reduction in **microbial electrosynthesis processes (MES)** [7-9] and water desalination in **microbial desalination cells (MDCs)** [10]. The main bottleneck of all these processes is the requirement of high current densities since electrons are either the desired product for MFCs, or the main driving force in MECs, MDCs and MES [11].

From the knowledge acquired by the use of these technologies, a new type of BES has been recently proposed to provide a novel means to control and stabilize the fermentation process, with the possibility to exceed metabolic limitations of balanced reactions. Indeed, fermentation processes are commonly used to produce different kinds of soluble molecules (e.g. alcohols or carboxylic acids), sometimes with a concomitant release of an energetic biogas containing hydrogen and/or methane. Fermentation is carried out by a large diversity of microorganisms, in pure or mixed cultures, that can use a wide range of substrates, including organic waste [12-13]. The main parameters affecting fermentative pathways include the type of microbial inoculum, the medium composition, pH, temperature, hydraulic retention time in continuous systems and accumulation of end products, *e.g.* the H_2 partial pressure.

Although all of these operational parameters have been intensively investigated, fine control and monitoring of a fermentation process in light of producing a specific product is very challenging especially when considering mixed cultures.

In this context, the **oxidation-reduction potential (ORP)** of the fermentation medium, also called extracellular ORP, appears to be a relevant parameter to control the microbial metabolism [14-15]. Indeed, a fermentation process corresponds to a cascade of oxidation and reduction reactions that must be kept in balance. These reactions are mostly thermodynamically favorable and spontaneous but they are also constrained by biological regulations within microorganisms and inter-species interactions in microbial communities. Similarly to pH as a measure of the protons activity, the extracellular ORP corresponds to the activity of the electrons present in the medium. It is mainly affected by temperature, chemical composition of the medium and the degree of reduction of the metabolites produced by fermentation. It can be easily measured with an ORP sensor located in the medium. The extracellular ORP is particularly important because it can subsequently affect the intracellular ORP through the **NADH/NAD$^+$** balance [16]. Intracellular ORP, representing the redox state inside a cell, can be estimated through the NADH/NAD+ ratio because of the intracellular redox homeostasis [16]. It is known to control gene expression and enzyme synthesis, impacting the whole metabolism that can further cause shifts in the metabolic pathways [16]. Chemical control of the extracellular ORP has already been successfully implemented to improve the production of metabolites such as succinate [17-18] or 1,3-PDO [19]. In this context, bioelectrochemical systems might be used to modify the extracellular ORP by

supplying or collecting energy in the form of an electric current through the presence of electrodes, in a process so-called **electro-fermentation (EF)**.

A novel type of BES: the electro-fermentation system (EFS)

Electro-fermentation principles

Electro-fermentation systems (EFS) could be defined as bioelectrochemical systems in which an electro-fermentation occurs to control self-driven fermentation (see Box 1). Electro-fermentation consists of operating the fermentation of an energy-rich substrate, such as a carbohydrate or an alcohol, in the presence of electrodes as supplementary electron source or sink. When the final product is more oxidized than the substrate (e.g. ethanol from glycerol), the **working electrode (WE)** would work as an anode and be used to dissipate the excess of electrons in an **anodic electro-fermentation (AEF)**. In contrast, for a reduced final product (e.g. butanol from glucose), the WE would supply electrons as a cathode in a **cathodic electro-fermentation (CEF)**. In this context, the electric current is not the product of interest nor the main energy source, but a trigger allowing the fermentation process to occur under unbalanced conditions. Moreover, in EF, the reaction is not only supported by the electronic current: even small current densities may affect both extracellular and intracellular ORP and thus the biological regulations through changes in NADH/NAD^+ balance that can significantly impact the final fermentation product pattern [20-31]. The main difference between EF and other BESs is that EF does not require high current densities to occur. To discriminate between these two processes, an electro-fermentation coefficient (η_{EF}) could be calculated (see Box 1). This parameter can also be used to estimate the energetic cost related to the production of a molecule of interest.

Terminology

As an emerging field of research, electro-fermentation has been investigated in only few studies and has not yet been well defined. Several terms have been used to describe this process, such as “unbalanced fermentation in microbial electrochemical cells” [21], “glycerol-fed bioelectrochemical system” [24], “bioelectrochemical fermentation” [25] or “electricity-driven biosynthesis” [26]. The concept and term of “electro-fermentation” was first proposed by Rabaey et al. [8] to designate this process. It was then used by several authors with the same meaning [20, 25, 32-33], but also to describe BES working as MFCs to produce H_2 and electricity from waste [34-37]. This lack of consensus may mislead the readers that are interested in this concept. To make more consistent this new way of using BES, we recommend the term “Electro-fermentation”. Conceptually, it is a clear way to designate a biological system that is driven first by the fermentative process, even though the metabolic pathways are influenced by the presence of electrodes.

Operational strategies for Electro-fermentation

The effectiveness of the EFSs will mainly depend on (1) the interactions existing between microorganisms, (2) dissolved redox couples of the medium, and (3) interactions between microorganisms and the surface of the electrodes through cellular mechanisms of **extracellular electron transfer (EET)**. Several strategies have been explored to ensure EET in electro-fermentation systems, as summarized in Table 1.

The use of pure cultures of electroactive microorganisms such as bacteria from the *Geobacteraceae* or *Shewanellaceae* families is of great interest because of their

ability to perform direct electron transfer with the WE [38]. Such microorganisms are able to grow as an electroactive biofilm and thus interact directly with the WE. However, only few microorganisms, such as *Clostridium pasteurianum* [20], are currently known to be both electroactive and able to consume a large range of carbohydrates or alcohols [11, 39]. To address this issue, co-cultures of electroactive and fermentative bacteria have been recently proposed to provide all the biological functions required for converting a substrate in electro-fermentation systems. As an illustration, such a strategy has been successfully applied with a co-culture of *Clostridium cellobioparum* and *Geobacter sulfurreducens* to produce ethanol from glycerol [22].

Interestingly, when none of the fermentative bacteria is electroactive, redox mediators such as neutral red [28] or methyl viologen [28-29] can be added to the fermentation medium and thus impact the extracellular ORP [21,28-31]. These chemicals can be oxidized or reduced by the fermentative bacteria and then recycled electrochemically at the electrode. They are here used as electron shuttles in a so-called mediated electron transfer [8, 38]. Another way to add a redox mediator in the case of a CEF is to produce H_2 at the cathode that could be further used as a one-way electron shuttle [23-26].

In addition, several authors proposed to metabolically engineer some fermentative bacterial strains of interest by adding the property of electro-activity. As an illustration, electron transfer in *Escherichia coli* was accelerated by 183% via a periplasmic heterologous expression of the c-type cytochromes CymA, MtrA and STC originated from *Shewanella oneidensis* [21]. In this case, however, the addition

of methylene blue as electron shuttle was required. Reciprocally, electroactive bacterial species can also be engineered to uptake and use a broader range of substrates. This approach was performed on *S. oneidensis* to stoichiometrically convert glycerol to ethanol, a biotransformation that cannot occur unless two electrons are removed via an external reaction, here through electrode reduction [27].

Although research is emerging in this field, all of these methods are extendable to mixed culture fermentation processes, as long as the initial medium or microbial community contains components or bacteria able to interact directly or indirectly with the electrochemical system [23-26].

Hypothetical mechanisms of electro-fermentation

The mechanisms underlying the different observations in EF are not always well described. Likely, more than one basic mechanism is involved (see Figure 1, Key figure).

Electron transfers and unbalanced fermentation

The electrodes present in the fermentation medium act like a non-soluble electron donor (cathode) or acceptor (anode) that is never limiting the reaction. Electron transfers between these electrodes and electro-active microorganisms can occur at the electrode surface through direct contacts or the presence of nanowires between the microorganisms and the electrode, or through extracellular polymeric substances produced by microbial biofilms [8, 38, 40]. Electron transfers can also be achieved without any biofilm formation through the presence of redox mediators either generated by fermentation, such as hydrogen, formate or acetate, or artificially

177 added such as methyl viologen [8, 38, 40] or neutral red [28]. These EET
178 mechanisms, well-described in the extensive literature dealing with the
179 characterization of anodic reaction in MFCs, are likely to be also those that can
180 occur during cathodic electron transfers [40].
181
182 In the context of EFSs, an immediate benefit of these EETs would be a direct
183 dissipation of excess electrons in AEF [27], or a direct conversion of a substrate into
184 a more reduced product in CEF [30-31] (see Figure 1A). Thus, CEFs would be a kind
185 of MES in which electrosynthesis would start from an electron-rich substrate instead
186 of CO_2 (e.g. 1,3-propanediol from glycerol). Ideally, the substrate would be
187 stoichiometrically converted into the desired product.
188
189 *Small current, high impact*
190 Even though such a conversion has already been observed [27,30-31], electric
191 current during EF is not always sufficient to explain the change in end products
192 distribution [20, 23, 26]. The η_{EF} (see Box 1) were estimated from electron balances
193 available in the different studies (see Table 1) and were often close to zero,
194 indicating that significant impact on fermentation patterns was observed with only
195 small current densities. For instance, Choi et al. [20] performed a CEF in which 0.2%
196 of the total electron input originated from the cathode. Considering a coulombic
197 efficiency of 100% and that all these electrons were used to produce butanol from
198 glucose, this would have led to a final butanol yield only 1.12-fold higher than the
199 fermentation control (see Figure 2). The observed butanol yield increase was
200 actually more than 3 times higher than the fermentation control, meaning that the

electrons used for the extra butanol production were mainly diverted from other metabolic pathways.

At a cellular level, the redox pairs homeostasis is crucial to ensure an optimal functioning of cellular metabolism [16, 41]. Several metabolic regulatory enzymes are known to specifically detect changes in extracellular and intracellular ORP, and adjust electrons flow in the metabolism accordingly through NADH/NAD+ ratio stabilization [41-42]. It is expected that the $NADH/NAD^+$ ratio might be affected by EETs with an electrode or soluble electron carriers as extra electron donor or acceptor [16]. In CEF operated with pure cultures, it was previously observed that more NADH was produced during EF when compared to the fermentation control. Choi et al. observed a $NADH/NAD^+$ ratio at the beginning of EF that was 5 times higher than the one obtained in fermentation controls [20]. In response to such an extra NADH, it was observed an increase of butanol production (net NADH-consuming) and a decrease of hydrogen and biomass production, with a final $NADH/NAD^+$ ratio similar than the one obtained in the fermentation controls [20]. This would indicate that cellular regulations resulting from unbalanced $NADH/NAD^+$ ratio have a stronger effect on metabolism than just a dissipation of the extra source of electrons (see Figure 1B) and, by extension, that other cellular mechanisms are involved. From a practical point of view, this would mean that EF can be performed with very low energy costs, resulting in a η_{EF} close to zero (see Table 1), albeit having high impact on the fermentation process. Also, in the cases EF was performed with redox mediators, a similar alteration of the $NADH/NAD^+$ ratio was observed, meaning that an electro-active biofilm is not always essential for such a mechanism to occur [21,28-29].

Syntrophic interactions

Although the use of pure cultures is of great interest in EFSs, supplementary benefits can be obtained from the use of mixed cultures of fermenters and electro-active bacteria. It was previously reported in MFCs that electro-active bacteria, able to perform anode respiration, are often associated in anodic biofilms with fermentative partners that can convert fermentable substrate into metabolites usable by the electro-active bacteria [43-47]. This relationship can be defined as syntrophic, as fermentative bacteria provide a substrate to electro-active bacteria that in return make the fermentation thermodynamically more favorable by removing its by-products [44]. The interactions between fermenters and electro-active bacteria rely on mechanisms of **interspecies electron transfer (IET)** either indirectly through the diffusion of electron carriers such as H_2, formate or other metabolites [47-48], or directly with the use of conductive pili [47-50], membrane to membrane contacts [47] or the presence of a conductive support on which a biofilm can attach [51-52]. These mechanisms usually occur in a biofilm in which contacts and interactions between microorganisms are favored. Such biofilms are spatially structured with electro-active bacteria being the most abundant organisms close to the electrode surface and fermenters dominating the top of the biofilm [22, 53]. It is worth mentioning that the biofilm thickness can be a limitation for those interactions to occur. By increasing the biofilm thickness, the diffusivity in the biofilm decreases, resulting in gradients within the biofilm (e.g. pH, redox mediators) and limitation of IET [54].

Even though they occur at a limited rate, these interactions are of huge interest for EFSs as they can provide a substantial support to fermentative bacteria (see Figure 1C) [55]. Indeed, when co-metabolites such as organic acids or H_2 accumulate in too high concentrations in the fermentation bulk or headspace, they often strongly inhibit

their own production and cell growth of fermentative bacteria, as observed in glycerol fermentation [22, 25]. Their consumption by electro-active bacteria in the biofilm through IET mechanisms both stimulate the fermentation process and increase the purity of the final product by removing undesired by-products [22, 56]. In this context, members of the *Geobacteraceae* family can be particularly preferred for their ability to consume several side-products of the fermentative pathways [39]. This mechanism is more likely to occur in an AEF because the electrons produced from the by-products oxidation can be transferred to the anode. However, it would also potentially exist in CEF if electrons are transferred from electro-active bacteria to fermenters through IET mechanism. Nonetheless the latter mechanism remains hypothetical and has never been proved in EF.

Mixed cultures

All of the mechanisms proposed above may also affect the selection of microbial populations when mixed cultures are used in EF. The addition of a driving force through a poised electrode creates an ecological niche that may favor the growth of electroactive bacteria and their partners in the form of a mixed biofilm whose microbial community is different from the planktonic community [23-25]. An indirect effect on population selection of planktonic bacteria would likely result in a significant effect on the final distribution of the fermentation products [25].

Concluding remarks and future perspectives

Thermodynamics is not the sole limitation in fermentation production yields, as most of the overall reactions that occur during fermentation are spontaneous. These

limitations are mostly due to biological regulations that keep the metabolism in a redox balance. The presence of an electrode inside the fermentation medium is a way to externally induce a shift from balanced to unbalanced fermentation, theoretically leading to a stoichiometric conversion of a substrate into a product of interest. Thus, EF presents the possibility of exceeding the theoretical maximum yields calculated for balanced fermentations, as shown *in silico* by Kracke and Krömer (2014) [32]. According to this simulation, many metabolites of economic interest, such as succinic acid or lysine, could be produced at significantly higher yields in EF compared to classic fermentation with very promising biotechnological outputs and could be good candidates for full-scale application of EF [32]. Such bioelectrochemical conversions will however require a relatively high current flow to ensure a good productivity, although lower than current consumed in MES, and therefore present similar limitations of most MFCs and MECs [33]. As stressed by Harnisch et al. [33], further fundamental research is needed and technological hurdles have to be taken.

Because it requires only little current flow (*i.e.* η_{EF} close to zero), the ORP control of the fermentation broth, mostly acting on the NAD^+/NADH balance, is for us the most promising mechanism to favor in EFS. It is an efficient way of controlling biological regulations that could lead to a more specific production of the desired end-product. The use of redox mediators makes it even more attractive since no specific interactions between fermenters and the WE is required. Thus, EF could be potentially applied as an additional control tool for any fermentation process. More specifically, it could be a solution to the most challenging issue of mixed cultures

processes, which is the increase of selectivity in fermentation patterns (i.e. production of a limited number of metabolites) and stability of this pattern.

EFSs also provide a new framework for the study of interactions between electro-active bacteria and fermenters in defined co-cultures as well as in mixed cultures in general. The external control of electrodes is an additional trigger that has an immediate and significant impact on electro-active bacteria and thus is an excellent tool to observe these interactions under well controlled conditions [44,46,57]. The material of the electrode is also a support that can lead to specific interspecies interactions such as direct electron transfer that would not be possible without a conductive surface available for electroactive bacteria attachment [51-52]. Since a growing number of electroactive bacteria have been discovered over the past years [5,39], new opportunities to observe specific interspecies interactions are emerging with new metabolic functions to be explored. This leaves a wide-open and exciting research field of new improved electro-fermentation processes (see Outstanding Questions), using a wide diversity of substrates, microbial catalyzers and targeted products.

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Figure Legend

Figure 1. Key Figure: Hypothetical mechanisms that can occur during anodic electro-fermentation. Mechanisms of cathodic electro-fermentation can be obtained by reversing all the electron fluxes. A: The substrate is directly converted into the product and the excess of electron is fully dissipated at the anode through mechanisms of extracellular electron transfer. B: The excess of electron generated during the oxidized products formation is not fully dissipated at the anode and part of the substrate is used for this purpose. Electron dissipation at the anode tends to decrease the $NADH_2/NAD^+$ ratio, resulting in regulations favoring one pathway to regenerate $NADH_2$. C: The fermentative microorganism (yellow) consumes the substrate but is not able to interact with the anode. The electro-active microorganism (red) acts as a mediator between the fermentative microorganism and the anode through mechanisms of interspecies electron transfer. The electro-active microorganism also consumes by-products from the substrate fermentation, favoring the whole fermentation process.

Figure 2. Comparison between a classical fermentation and an electro-fermentation. The values in percentage represent the initial electron contribution (substrates) or the electron recovery (products) obtained during experiments performed by Choi et al. [20]. Adapted from Harnisch et al. [33].

Glossary

Anodic electro-fermentation (AEF): Electro-fermentation in which the anode is the working electrode.

Bioelectrochemical system (BES): Electrochemical process in which at least one reaction is catalyzed by microorganisms or enzymes.

Cathodic electro-fermentation (CEF): Electro-fermentation in which the cathode is the working electrode.

Electro-fermentation (EF): Self-driving fermentation operated in the presence of polarized electrodes as a driving tool.

Electro-fermentation system (EFS): Cells in which electro-fermentation is performed.

Extracellular electron transfer (EET): Mechanism that allows electron transfer from a microorganism to an extra-cellular electron acceptor (anodic EET) or from an extra-cellular electron donor to a microorganism (cathodic EET).

Interspecies electron transfer (IET): Mechanism that allows electron transfer between different species of microbes. This transfer can be either direct or mediated by electron shuttles.

Microbial desalination cell (MDC): BES(s) used for desalination.

Microbial electrolysis cell (MEC): BES in which substrate oxidation is combined with the addition of a small voltage to enable hydrogen gas evolution or other energetically unfavorable biological/chemical reactions at the cathode [7, 58].

Microbial electrosynthesis (MES): Execution of microbially catalyzed electrochemical reactions to transform a substance into a desired product [59].

Microbial fuel cell(s) (MFC): BES that convert energy, available in a bio-convertible substrate, directly into electricity [5].

Nicotinamide adenine dinucleotide reduced/oxidized (NADH/NAD^+): Cellular electron carrier.

Oxidation-reduction potential (ORP): Correspond to the tendency of a solution to either gain or lose electrons.

Working electrode (WE): Electrode on which the working potential is applied.

Box 1: Electro-fermentation characteristics and efficiency

The simplest definition of electro-fermentation is “a self-driving fermentation operated in the presence of polarized electrodes as a driving tool”. However, it can be sometimes difficult to assess whether a BES can be considered as an EF from this straightforward definition. As an illustration, Nikhil et al. performed a glucose fermentation in a BES designed for this purpose [34]. The aim of this process was to convert glucose into both electricity and hydrogen, with electricity as the main product. Electric current production represented between 40 and 70% of the initial electron input whereas 5 to 25% of it were used for hydrogen production. The aim was not to use electrodes as a tool to influence metabolic pathways of glucose fermentation but to get the most efficient electron recovery through electricity production, as in a MFC. Therefore, the process cannot be considered as an EF.
To clarify the conceptual limits of EF, it is necessary to define new indicators that would help in discriminating EF from other BESs. To do so, we propose to calculate an “Electro-fermentation coefficient”, analogous to the Coulombic efficiency, as follows:

$$\eta_{EF} = \frac{Q_{e-}}{Q_{product}}$$

Where η_{EF} is the electro-fermentation efficiency, Q_{e^-} the charge that was transferred through the electric circuit, $Q_{product}$ the total charge in the product *i.e.* the charge that would be produced by a total oxidation of the desired product.
Q_{e^-} is easy to calculate from chronoamperometry. It is the integral of the electric current (I) over the time of the EF operation:

$$Q_{e-} = \int I \, dt$$

To calculate $Q_{product}$ it is first necessary to calculate the number of moles of electrons available per mole of product ($N_{product}$), as follows:

$$N(C_w N_x O_y H_z) = 4w - 3x - 2y + z$$

Then, noting $n_{product}$ the number of moles of product of interest and F the Faraday constant (96,485 C / mol_{e^-}), $Q_{product}$ can be calculated as:

$$Q_{product} = n_{product} . N_{product} . F$$

The value of η_{EF} indicates if electricity production or consumption is predominant over the production of the molecule of interest during EF. More specifically, if its value is between 0 and 1, more electrons will be recovered in the product than those provided (AEF) or consumed (CEF) to/from the electric circuit. If its value is over 1, then it is likely that an “AEF” is in fact close to a MFC (electricity production), or that a “CEF” is actually close to a MES (electrosynthesis). Therefore, it can be a relevant parameter to be used to assess EF energetic performances.

545 **Table 1: Electro-fermentation applications and operating parameters**

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Inoculum	Substrate	Aimed final product	Working potential (V *vs.* SHE)	Redox mediator	η_{EF}*	Improvement *vs.* fermentation control	Ref.
Anodic electro-fermentation							
Engineered *Shewanella oneidensis*	Glycerol	Ethanol	0.40	No	0.25	No fermentative control	[27]
Clostridium cellobioparum *Geobacter sulfurreducens*	Glycerol	Ethanol	0.46	No	0.03	Acetate, H_2 and formate removal Increased glycerol consumption	[22]
Engineered *Escherichia coli*	Glycerol	Ethanol Acetate	0.20	Methylene blue	0.02	Increased glycerol consumption rate	[21]
Cathodic electro-fermentation							
Clostridium pasteurianum	Glucose	Butanol	0.045	No	0.01	3-fold increase in butanol production yield	[20]
Clostridium acetobutylicum	Glucose	Butanol	NA	Methyl viologen	NA	26% increase in butanol production yield	[29]
Clostridium tyrobutyricum	Sucrose	Butyrate	-0.17	Neutral red	NA	30% increase in butyrate production yield	[28]
Propionibacterium acidi-propionici	Lactose	Propionate	-0.47	Cobalt sepulchrate	0.10	No acetate/lactate production. Propionate was the only product.	[30]
Propionibacterium freudenreichii	Glucose	Propionate	-0.39	Cobalt sepulchrate	0.15	No acetate production. Propionate was the only product.	[31]
Clostridium pasteurianum	Glycerol	1,3-propanediol	0.045	No	0.01	2-fold increase in 1,3-propanediol production yield	[20]
Mixed culture	Glycerol	1,3-propanediol	-0.90	No	0.34	2-fold increase in 1,3-propanediol production yield	[26]
Mixed culture	Glycerol	1,3-propanediol	~ -0.80†	No	0.05	No fermentative control	[23]
Mixed culture	Glycerol	1,3-propanediol	~ -1.44†	No	0.38	No fermentative control	[23]
Mixed culture	Glycerol	-	~ -1.28†	No	NA	Increased glycerol consumption	[24]

547 * Electro-fermentation efficiency estimated from mass an electron balances available in the different
548 studies.
549 † Bio-electrochemical reactors operated with an imposed electrical current
550

Trends

- With the aim of producing organic molecules from complex substrates and being economically competitive, fermentation processes are constantly optimized to reach higher yields, higher production rates or a higher selectivity in metabolic end-products. The use of genetically modified microorganisms has proved their efficiency besides having high operation costs due to obligatory sterile conditions and very restrictive legislation. In contrast, mixed culture fermentation processes present low operation costs and can deal with more complex substrates, but suffer from lower conversion yields and a lack of selectivity in terms of fermentation end-products.

- To address these issues, electro-fermentation is a recent technology that can be used as new driving tool. Expected impacts on conventional fermentations are to enhance and better control the microbial fermentation by increasing the specificity of the metabolic routes and overpass the thermodynamic limits.

    - First results in electro-fermentation have been reported with both pure and mixed culture systems. The first electro-fermentation observations were very promising with high impacts on the fermentation patterns despite the low current densities. As an illustration, a significant enhancement of the production of specific metabolic end-products such as 1,3-propanediol or butanol were observed, making ‘electro-fermentation’ a new and very promising field of investigation in the domain of fermentation.

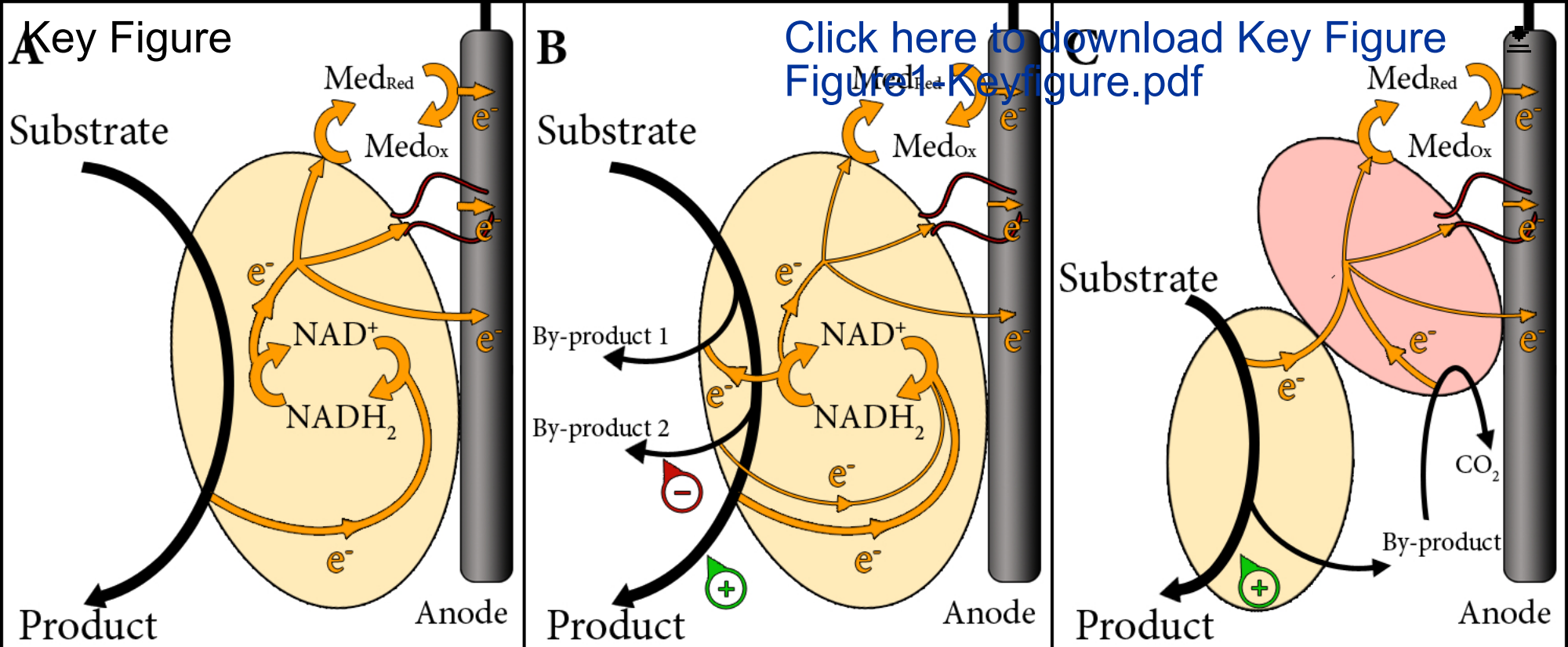
Outstanding Questions

- What is the most efficient strategy that should be developed to maximize the impact of electro-fermentation on metabolic patterns in terms of selectivity or fermentation yields?

- Would electro-fermentation make mixed or co-cultures economically more competitive against processes using genetically modified microorganisms? Since specific bacterial selection has been observed in mixed cultures electro-fermentation compared to conventional fermentation, can electro-fermentation systems be used to finely select efficient microbial consortia?

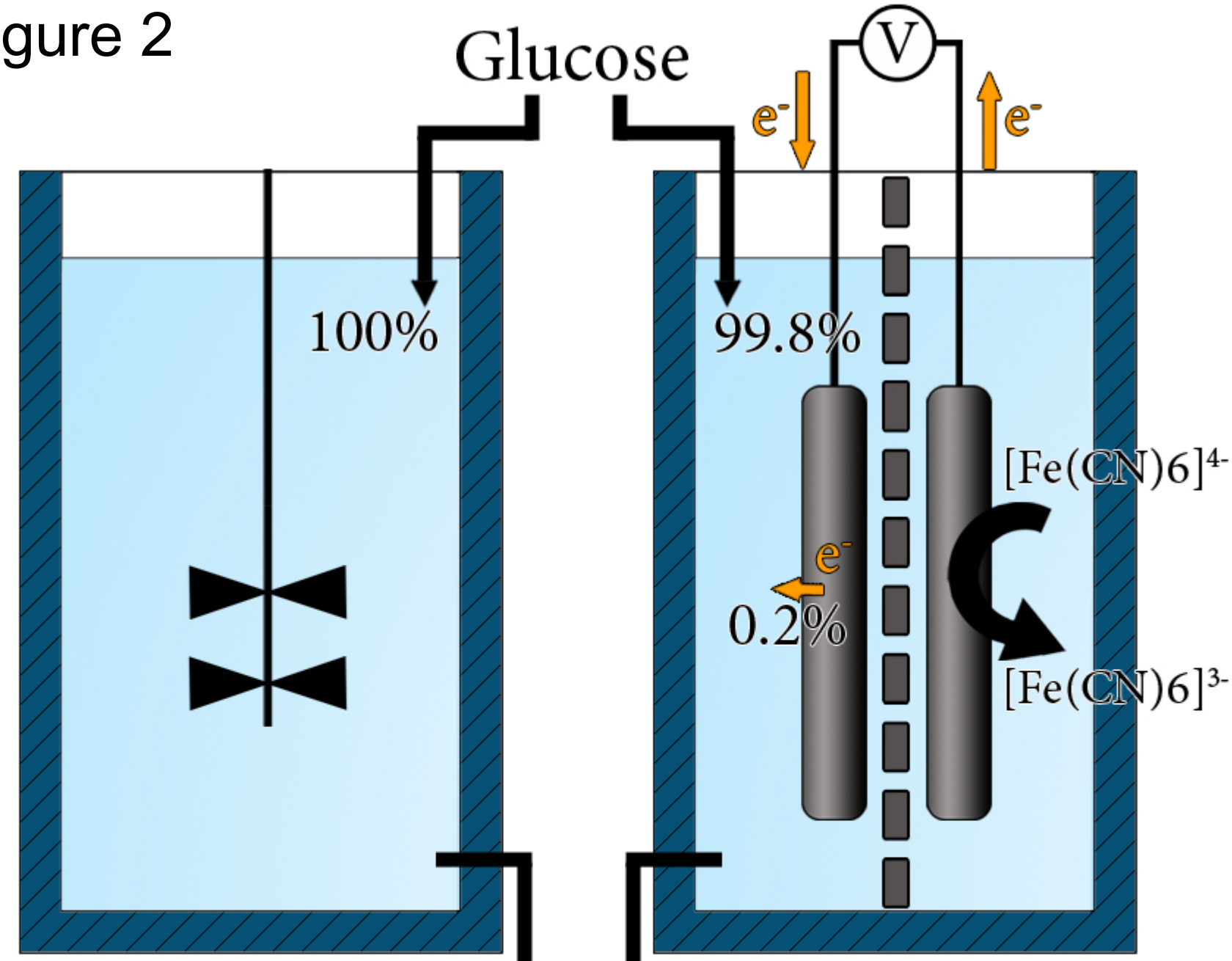
- Recently, electro-activity has been successfully added to fermentative microorganisms by genetic modifications. When associated with electro-fermentation, would these modifications increase the production yields beyond the current theoretical maximum? What are the opportunities of electro-fermentation to open a new field of investigation in metabolic engineering by concomitant optimization of both fermentative and electro-active systems?

- So far, only a few experiments have shown the electro-activity of strict anaerobic fermentative bacteria. Is the ability to interact with an electrode widely spread in anaerobic bacteria that could be directly exploited?



Carbon flux Electron flux Disfavored pathway Favored pathway

Figure 2



electron recovery	5%	Butanol	16%
	15%	Biomass	10%
	9%	Hydrogen	6%
	13%	Acetate	13%
	42%	Butyrate	41%