# 2017 年臺灣國際科學展覽會
# 優勝作品專輯

作品編號 **070011**

參展科別 **微生物學**

作品名稱 **A Novel Selection Process for the Conversion of Conventional Bacteria into Electrotrophs**

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# 作者照片

# Abstract

**Background:**

The redox reactions of bacteria metabolism have been extrinsically studied. These mechanisms allow certain types of bacteria to be able to synthesize extremely valuable extracellular byproducts. Other types of bacteria are able to extract toxic metals from water by donating electrons directly to those aqueous metal ions, thus turning them into solid precipitates. However, the problem of these microorganisms is that their efficiency rates and production speeds are exceptionally low. This study focuses on the properties of electrotrophs, which are bacteria that can feed on pure electrons directly from an electrode (Rabaey et al 2010). Compared to normal organic-feeding bacteria, electrotrophs direct the majority of the electrons obtained to the production of metabolic byproducts (Nevin et al 2010). Therefore, when electrotrophs are employed in bioelectrochemical systems (BESs) their metabolic redox reaction efficiency rates are dramatically increased. This makes it possible to produce large quantities of valuable compounds such as hydrocarbons, plastics and medicine or efficiently remediating the environment (He et al 2016). Moreover, the usage of electricity as an energy source compared to conventional organic substrates is immensely cheaper (Rabaey et al 2010). However, not all bacteria are electrotrophs nor do all electrotrophs have favourable metabolic traits. Thus, there is a need for a novel procedure to turn conventional bacteria into electrotrophs which is a crucial step to making the BES an aggressive competitor in the sustainable energy industry.

**Purpose:**

The purpose of this project is to turn E. coli K12 (non-electrotrophic bacteria) into an electrotroph. These investigations will shed light on methods to improve current BESs and will help develop further applications for this ever-advancing technology.

**Procedure:**

**Overview:** Three groups of BESs were tested in identical conditions with identical catholyte and anolyte solutions. The first group was a sterile BES. The second group of BESs were only inoculated with wild-type E. coli while the third group of BESs were inoculated with a mutant batch of E. coli. Through multiple enrichment and selection processes, electrotroph strains were selected and isolated. Current of all three groups were recorded and analyzed. Pure electrotrophs strains were then isolated to analyze pure culture current production.

**BES Construction:** A two chamber BES was constructed with a carbon cloth electrode, titanium wire, proton exchange membrane, rubber gaskets, injection stoppers, steel bolts and nuts, rubber washers and polypropylene sheets. See figure 1.1 and 1.2.

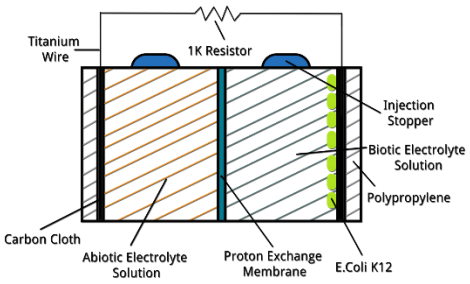


*Figure 1.1 BES configuration and schematic*

**Bacteria, Media and Solutions:** Escherichia Coli K12 inoculated in Luria broth (LB) was used in all biotic experiments. A 254 nm UV lamp was used to create a mutant pool of E. coli. Potassium ferricyanide and phosphate buffer saline (PBS) was used as the catholyte when a bioanode was run. Potassium ferrocyanide and PBS was used as the anolyte when a biocathode was run.

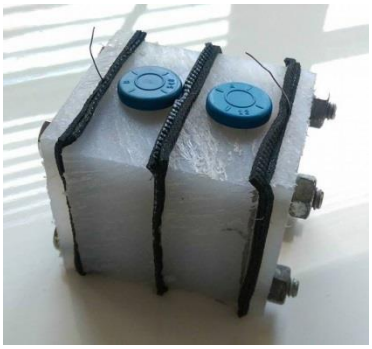

*Figure 1.2 Fully assembled BES*

**BES Operation:** The BES ran for 96 hours with current measured over a shunt resistor using a data voltage logger (Dataq DI-149). Every 16 hours, 50% of the electrolyte in both chambers were discharged and a sample was taken from the "electro-active" bacteria selective bioelectrode, which was placed in 10mL of LB. The sample was used to inoculate the next discharge. The BES was then refilled with fresh electrolyte solution and reran.

**Biofilm Enrichment Procedure:** Carbon cloth electrodes were first inoculated in mutant E. coli rich LB for 16 hours to speed up the biofilm growth process. The startup of biocathodes is extremely difficult and time-consuming compared to the startup of bioanodes (Zaybak 2013). Thus to speed up this process, bioanodes were first grown and then "reversed" into biocathodes. Electricigenic bacteria (able to extracellularly donate electrons to an electrode) often are also electrotrophic (able to extracellularly accept electrons from an electrode). Therefore, for the first 96 hours, potassium ferricyanide was used as an electron acceptor in the cathode chamber which allowed for the selection and growth of a stable electricigenic biofilm on the anode. The potassium ferricyanide was then replaced with potassium ferrocyanide - an electron donor - which reversed the polarity of the BES, causing the bioanode to turn into a biocathode and initializing the start of electrotrophic selection.

**Electrotroph Selection and Analysis:** Once power output growth slowed. Samples were taken from the biocathode. The samples were then streak-plated to isolate individual colonies. Nine colonies were chosen at random and grown in a BES to analyze pure culture power output.

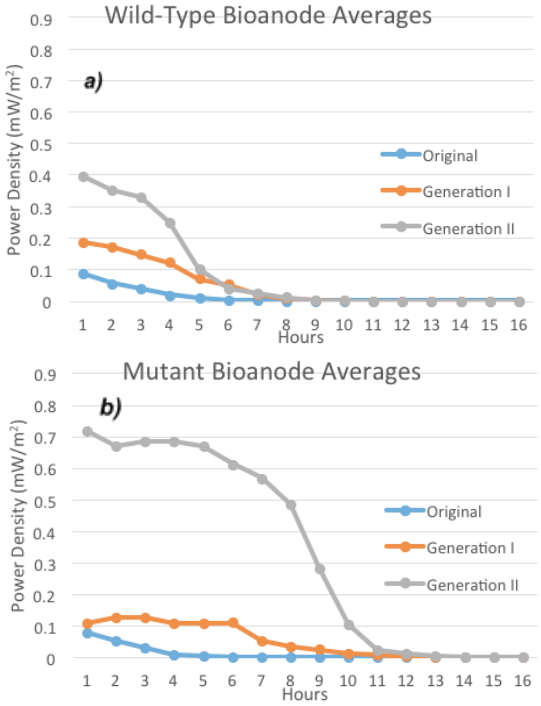


***Figure 2. Average bioanode power output of a) wild-type BES b) mutant BES***

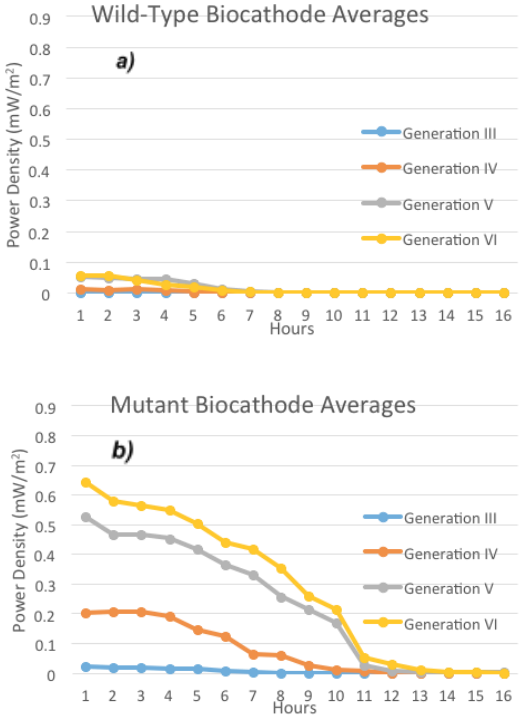


***Figure 3. Average biocathode power output of a) wild-type BES b) mutant BES***

**Results:**

**Data Analysis:** Power output was determined using the MFC power density equation: $P_{cat} = E_{cell}\, I/A_{cat}$ Where $P_{cat}$ is the power density, $E_{cell}$ is the cell voltage, I is the current and $A_{cat}$ is the area of the cathode (Logan et al 2006). Both the wild-type strain BES and the mutant strain BES showed improvement in electron transport efficiencies. The abiotic BES displayed no growth, showing that the chemical makeup of the BES did not influence power output growth. E. coli K12 has a below average mutation rate - 0.001 mutations per genome per cell generation - (Lee et al 2012), making it highly unlikely that an electrotrophic strain would appear in the wild-type BES. It can then be deduced that the wild-type strain went through epigenetic changes which increased power output (Fig. 2a & 3a). The mutant BES was more efficient than the wild-type BES in both the bioanode and the biocathode runs (Fig. 2 & 3) indicating a successful selection of electrotrophs. After the polarity of the BES was reversed and the bioanodes turned into biocathodes, it can be seen that the mutant BES had a higher power output than the wild-type mutant on its first run (Fig. 3: Generation III). This shows that the bioanode growth phase selected for electricigens and that they share traits and mechanisms with electrotrophs.

**Pure Electrotroph Strain Analysis:** Sample 3 displayed the highest power output with a peak of 0.258 $mW/m^2$ (Fig. 4). This is significantly lower than the power output peak in the mixed mutant BES (0.645 $mW/m^2$) (Fig. 3b), however this is expected. BESs operating with a mixed culture achieve power densities substantially higher than pure cultures (Logan et al. 2006), due to collaboration between the different strains. The average peak power output of all strains was 0.118 $mW/m^2 \pm 0.093$ $mW/m^2$.

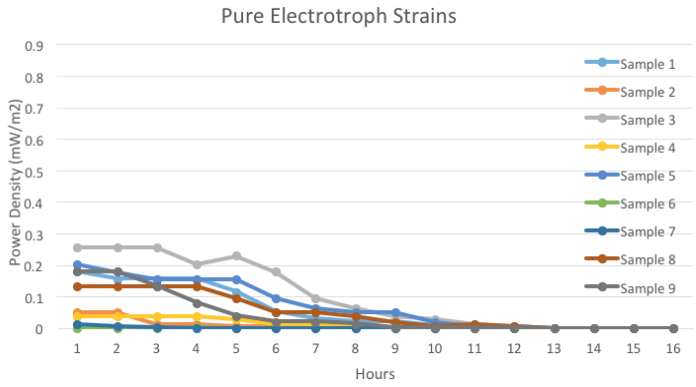


***Figure 4. Power density of pure electrotroph strains***

**Real Life Applications:**

The novel procedure created allows normal bacteria to be turned into electrotrophs. This increases the bacteria's natural metabolic redox reactions rate which means valuable compounds can be synthesized and that toxic metals can be extracted both faster and cheaper. Moreover, the isolation of electrotrophic strains of bacteria from mixed cultures is a time-consuming and sometimes impossible task, due unstable biocathode growth. However, this procedure solves those issues and acts as an isolation method for electrotrophic strains which can lead to the discovery of novel electrotrophs. The methods outlined in this study definitely is a crucial stepping stone for the further advancement of bioelectrochemical technologies.

**Conclusions:**

After conducting these experiments, it can be concluded that electrotroph strains were successfully selected for in a BES. It can be clearly seen that each "electro-activated" generation was more efficient at transporting electrons than the next by the increased power output. The insights gathered from this study paves the way for further improving BES efficiencies and allow bacteria with special metabolic systems to be turned into electrotrophs for mass chemical production or for specific chemical extraction from water (Pant et al. 2012).

**Acknowledgements:**

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**Appendices:**

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## 【評語】070011

1. UV light inducing mutation of E. coli K-12 results in the conversion of conversional Bacteria into Electrotrophs. The author provided the interesting data to achieve her goals.
2. If the author can use whole-genome sequencing of E. coli and identify the possible mechanism for this conversion the application of this study will be useful.