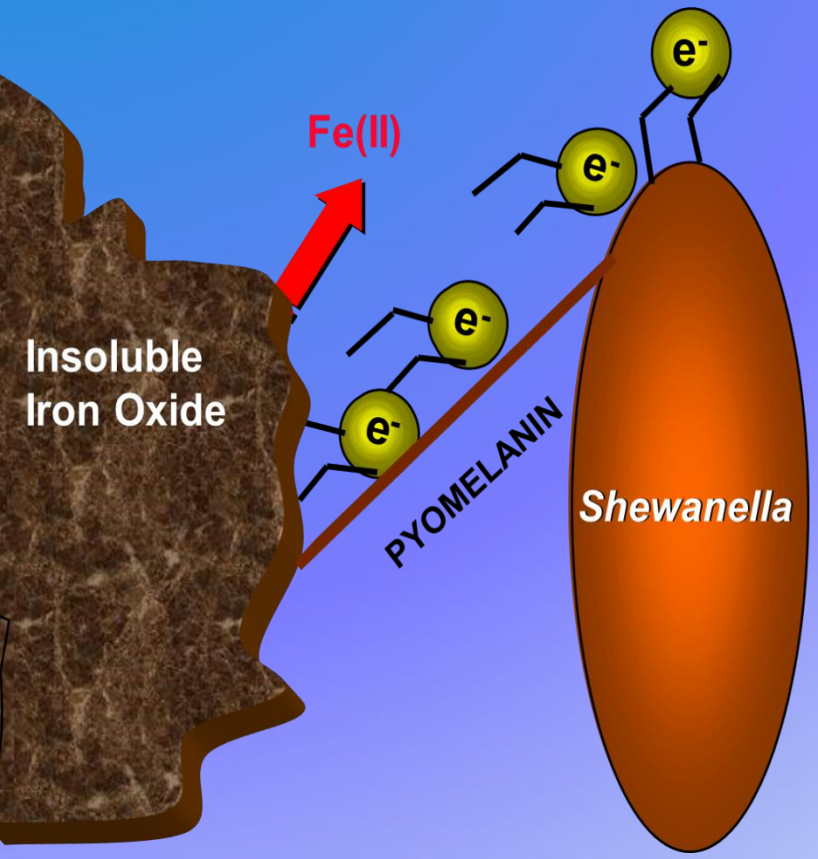
Adventures in Microbial Electron Transfer and Technology Development



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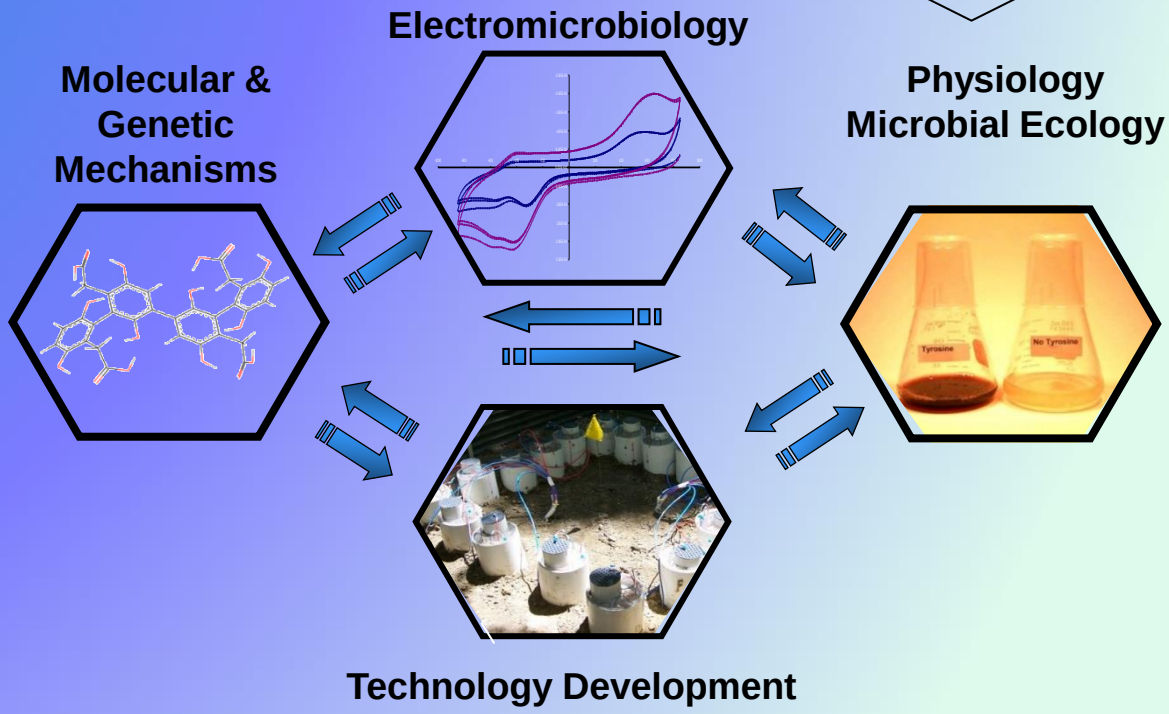
Progress to Technology Development

Fundamental Science → Applied Science → Technology Development

New scientific information moves from fundamental science to potential applications and then ultimately to technology development.

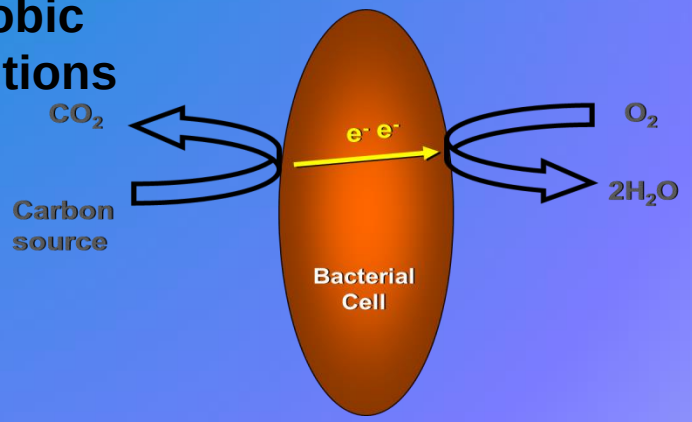
This process is not linear, but is very iterative. Often as we learn more about a specific application, we are better able to direct new fundamental studies.



The following slides highlight research directed at understanding how bacteria change the chemistry of toxic metals. This is useful for biotechnology development for detoxifying contaminated environments. This work is also leading to new applications from microorganisms that transfer electric current as well as bio-inspired radiation resistant materials.

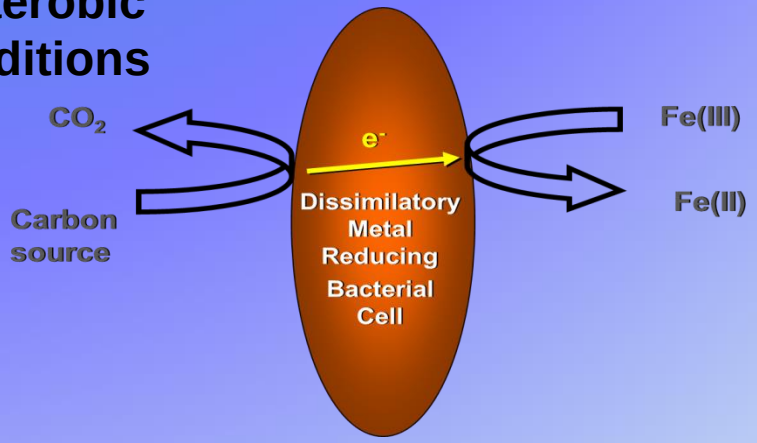
Aerobic and Anaerobic Respiration

Aerobic conditions



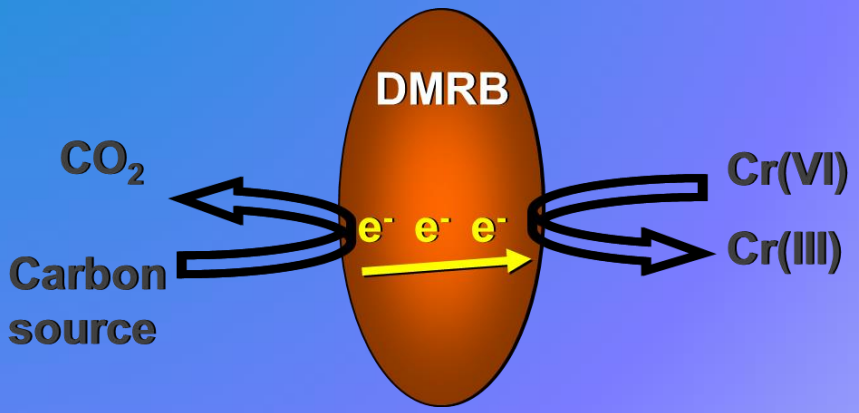
Under aerobic conditions many bacteria can use oxygen as a terminal electron acceptor to couple growth to energy conservation.

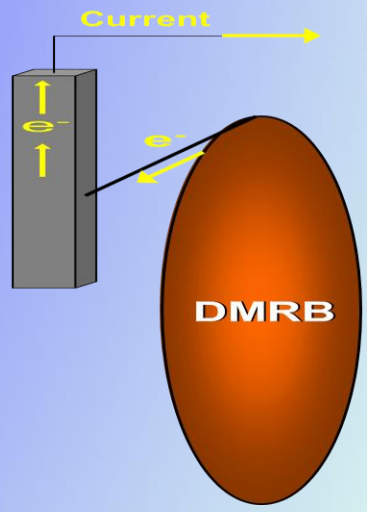
Anaerobic conditions

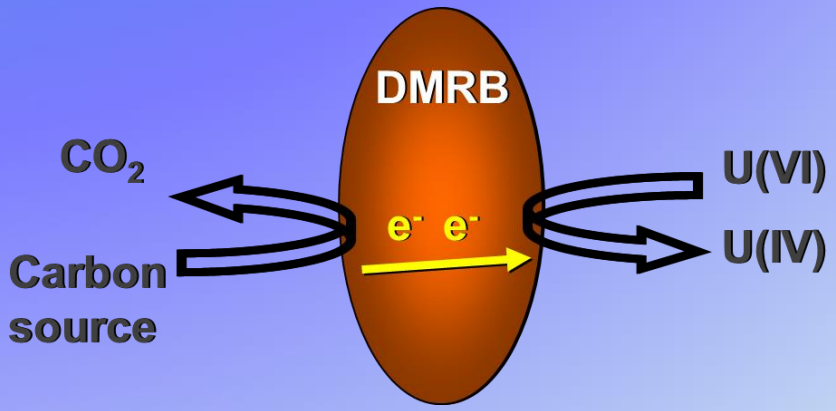


In the absence of oxygen, respiration is still possible with many bacteria. A common anaerobic terminal electron acceptor is Fe(III). Fe(III) oxides and dissimilatory metal reducing bacteria (DRMB) are common and can play important roles in environmental cleanup and biotechnology.

Applications of DMRB in Biotechnology

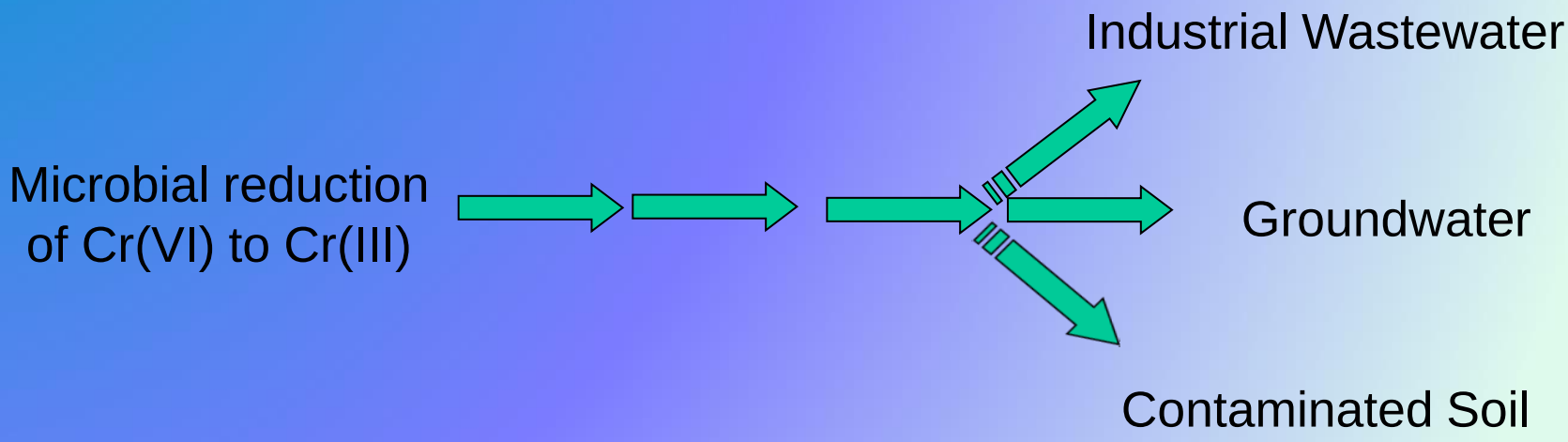






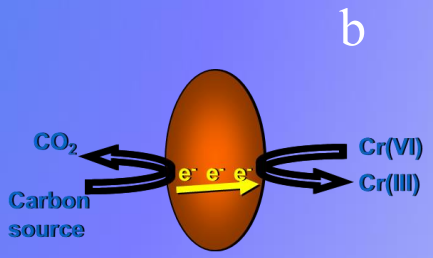
DMRB can be used to detoxify environmental contaminants like hexavalent chromium and uranium. The ability of DMRB to transfer electrons to solid terminal electron acceptors (like electrodes) also creates opportunities to study microbes with electrochemistry known as electromicrobiology.

Challenge: Understand How Bacteria can be Used In a Biotechnology for Cr(VI) Reduction for Detoxification

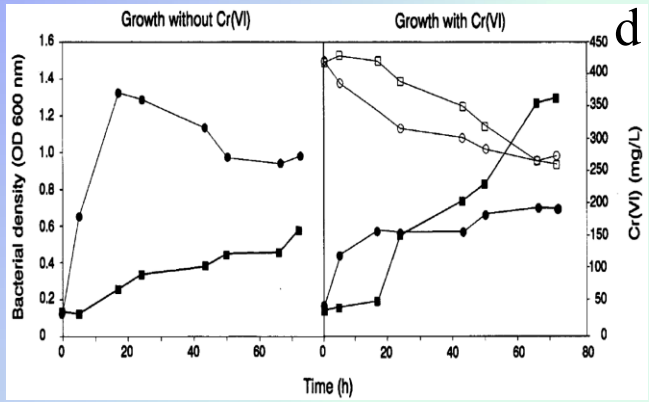


The goal was to develop a biotechnical approach employing bacteria to chemically reduce toxic, soluble Cr(VI) to the much less toxic and less soluble Cr(III). Industrial collaborators had simple operational requirements; turn it on, plug it in and walk away. This meant that the bioprocess could not be complex, like the use of pure cultures. Instead the technology had to rely on microbial ecology and incorporate robust and adaptive cultures.

Establishing the Ubiquity of Cr(VI) Reducing Bacteria



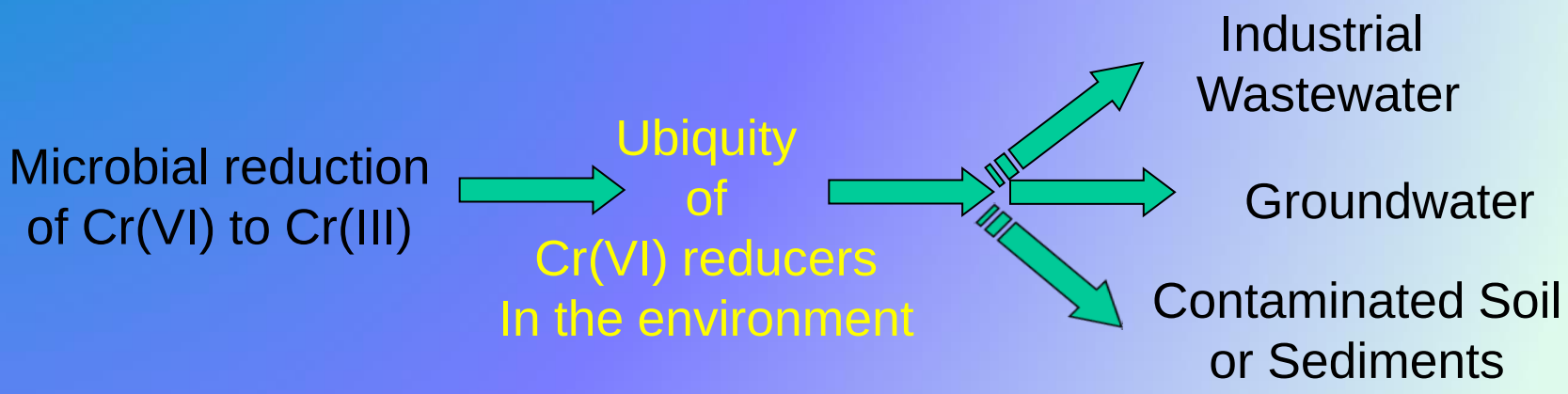
Isolating Cr(VI) reducing cultures from contaminated environments (a) was the first step to show that some environmental bacteria can adapt to use Cr(VI) as a terminal electron acceptor (b). Demonstrating that Cr(VI) reducing bacteria can be selected from pristine environments (c) showed that Cr(VI) resistance and reduction is common and bacteria from any environment can be used in a robust Cr(VI) reducing bioreactor (d).



Appl.Microbiol. Biotechnol. 44:683-688
J. Environ. Eng. 124:449-455

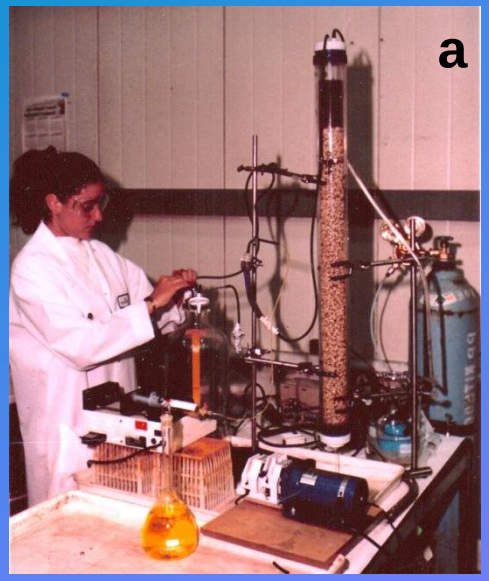
Biotechnol. Lett. 19:691-694
Appl. Biochem. Biotechnol. 63-65:855-864

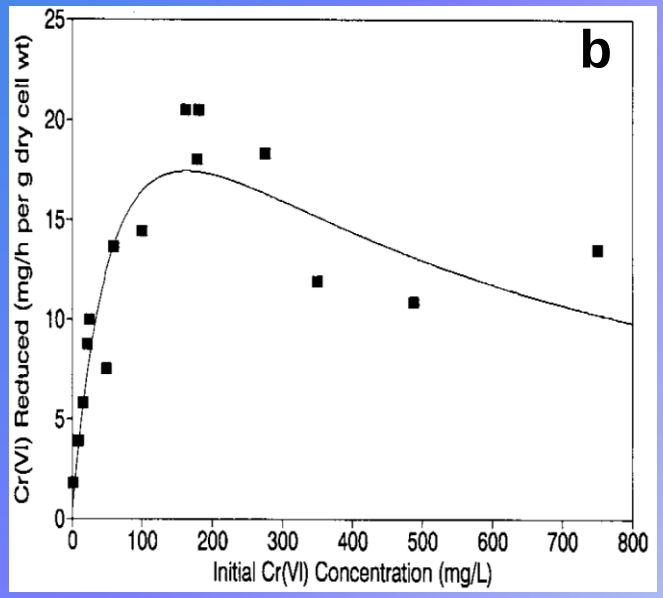
Challenge met: Exploiting the ubiquity of Cr(VI) reducing bacteria provided a foundation for technology development

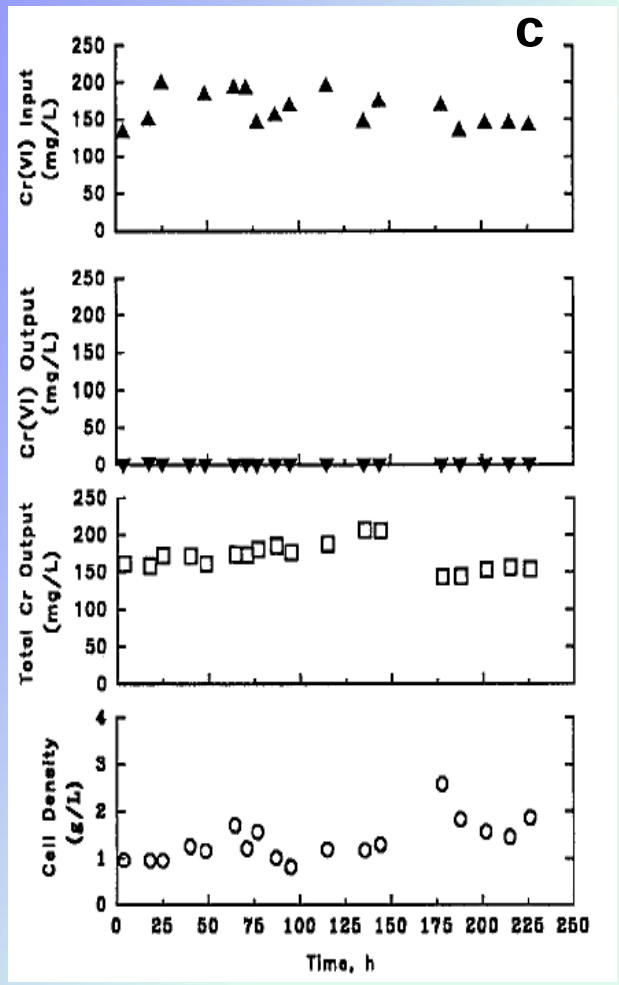


The discovery that Cr(VI) reducing bacteria are common in the environment allowed us to develop a bioprocessing strategy where we allowed a Cr(VI) environment to select for Cr(VI) reducers. Non Cr(VI) reducers were out competed. So, pure cultures are not needed and the microbial community is self regulating.

Microbial Ecology Studies and Bioreactor Proof-of-Principal



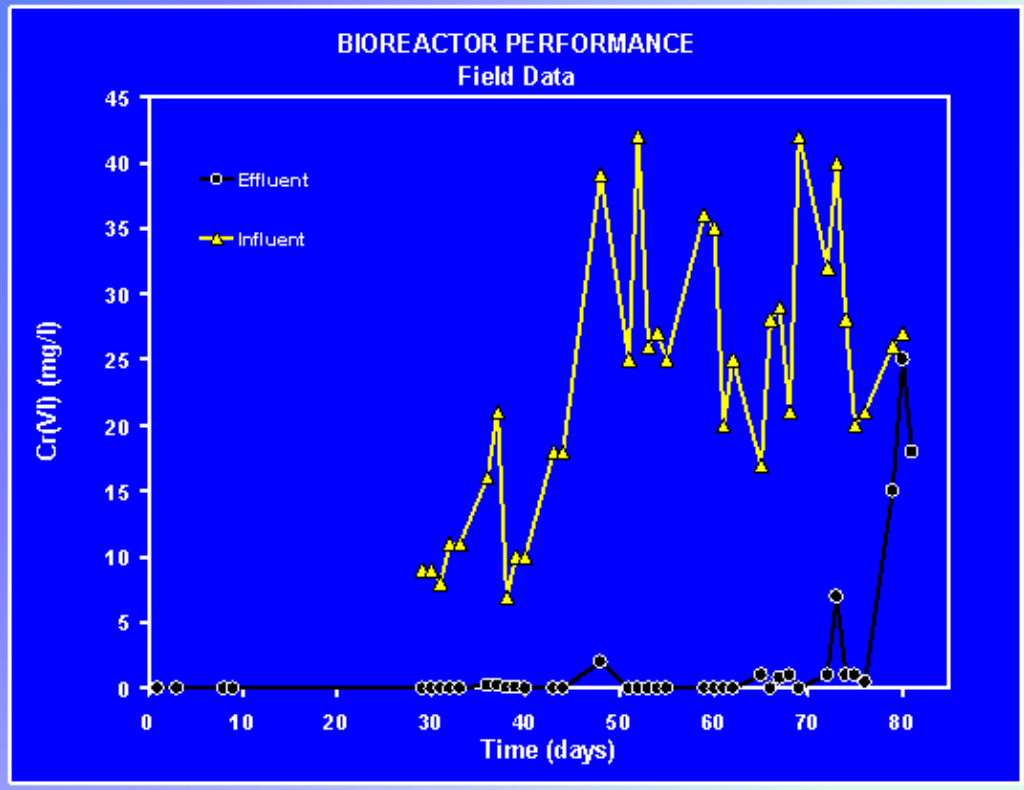




Incorporating a mixed culture of Cr(VI) reducing bacterial biofilm into a bioreactor (a) demonstrated that a robust mixed culture could be isolated from the environment. The mixed culture biofilm grew well across a wide range of Cr(VI) concentrations (b) and reduced about 200 mg/l of Cr(VI) with a 48 hr. retention time (c).

J. Ind. Microbiol. Biotechnol. 18:247-250

Microbial Ecology Studies and Bioreactor Field Study

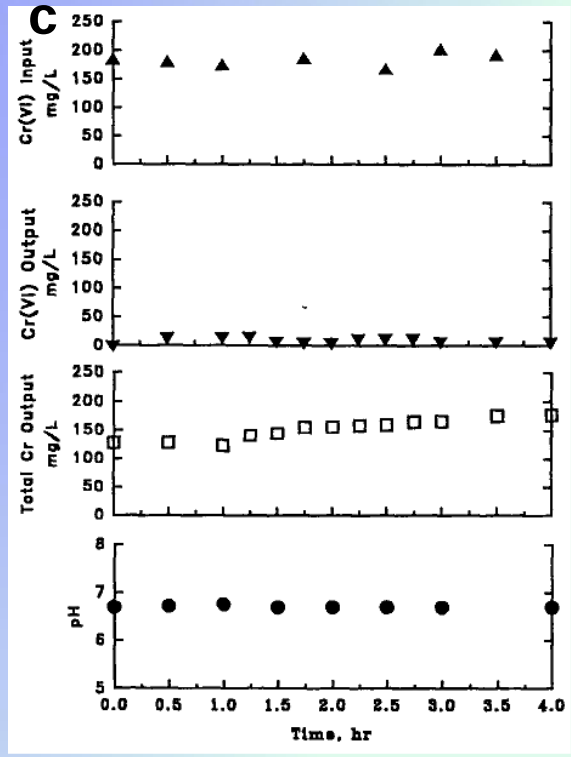


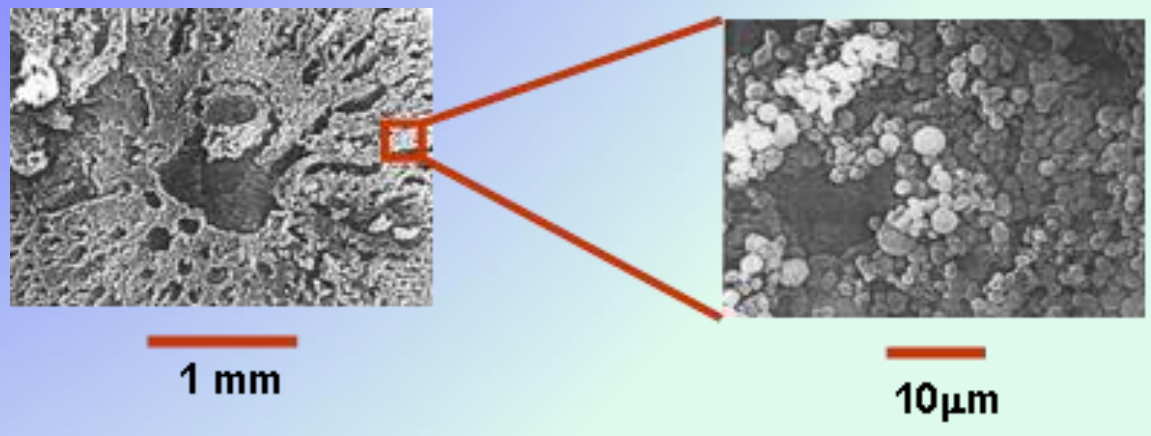
Our technology was incorporated into a 30,000 liter industrial bioreactor to remove Cr(VI) from waste leachate at a chromium steel factory in Sweden. Indigenous Cr(VI) reducing bacteria dominated the bioprocess that was fed acetate waste from a neighboring industry. The resulting Cr(III) precipitated inside the bioreactor as a hydroxide.

Vatten 53:245-251

High Throughput Bioreactor Study

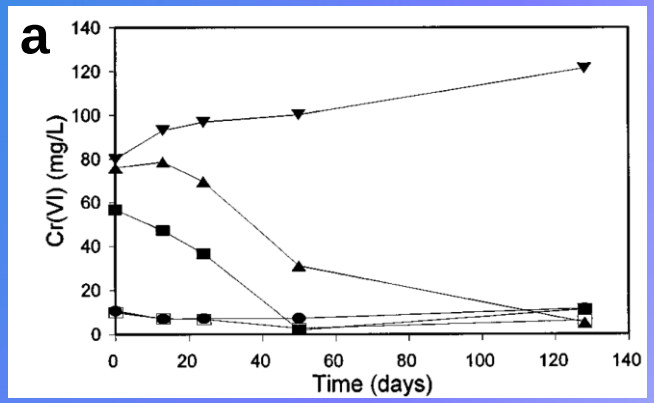
A high throughput bioreactor (a) was developed in order to treat industrial effluents with low concentrations of Cr(VI). Immobilized cell technology was used to increase cell density in the bioreactor and maintain low cell density in the effluent (b). This resulted in an increase in volumetric productivity (c) and low BOD in the bioreactor effluent.



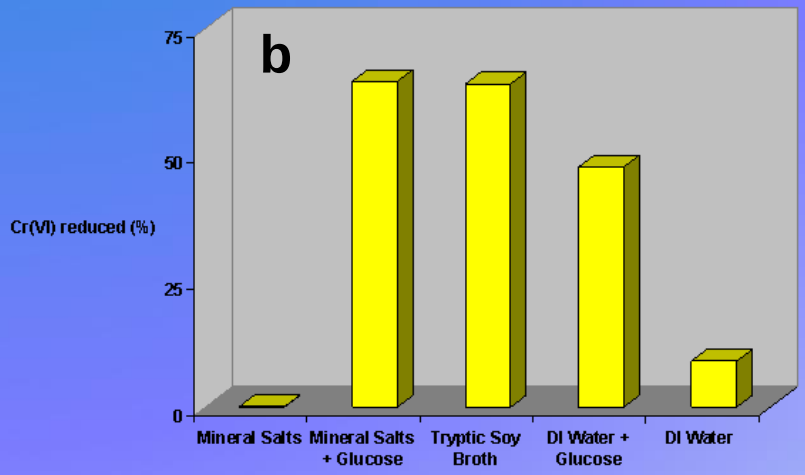


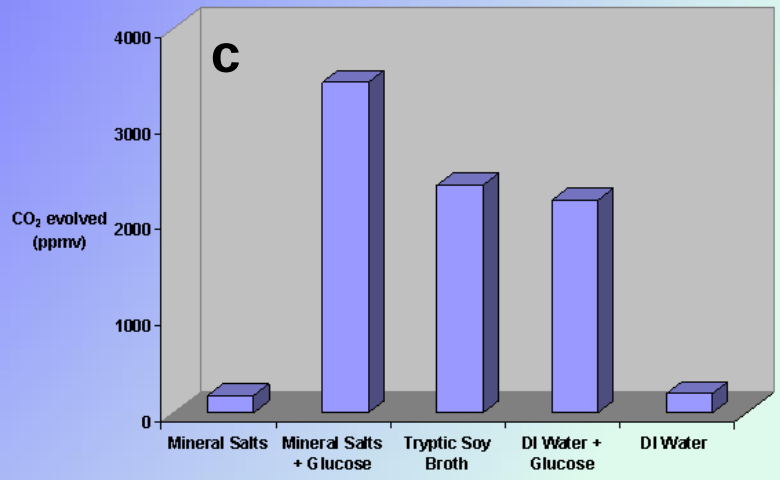
Appl. Biochem. Biotechnol. 63-65:871-877

In-Situ Soil Bioremediation Demonstration



- ▼ Mineral Salts
- ▲ Mineral Salts + Glucose
- ■ Tryptic Soy Broth
- ● DI Water + Glucose
- ○ DI Water

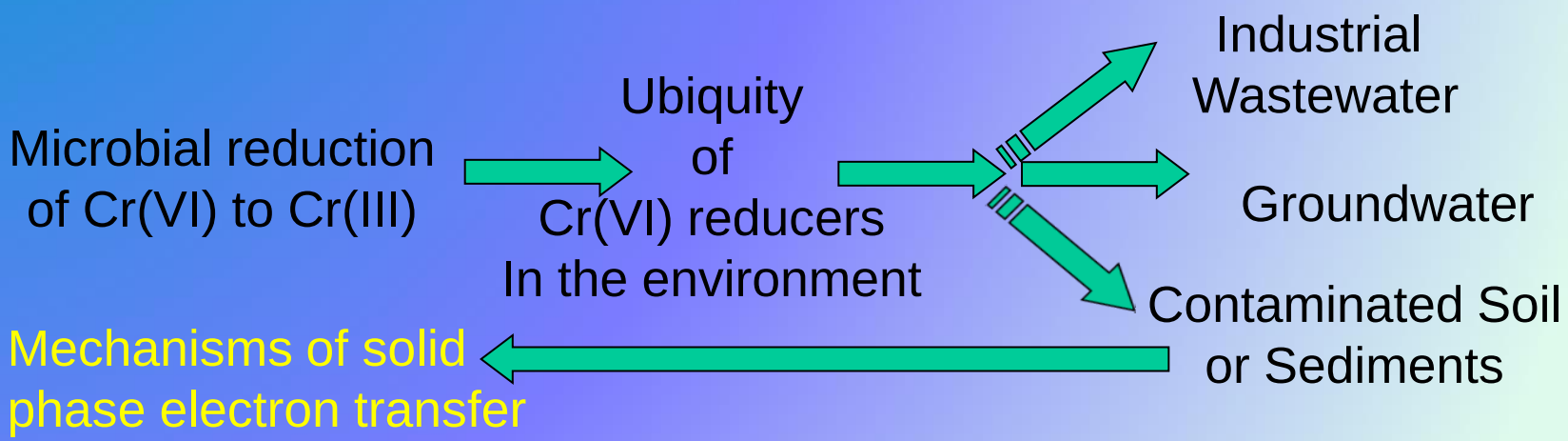




Carbon and energy sources added to Cr(VI) contaminated soil (a) allowed indigenous bacteria to detoxify the soil (b) in relation to bacterial growth (c). Some of the nutrient supplements to the soil caused Cr(VI) to desorb from soil particles. This showed that Cr(VI) in solution is more bioavailable and was reduced faster by bacteria compared to Cr(VI) sorbed to soil minerals (solid phase Cr(VI)).

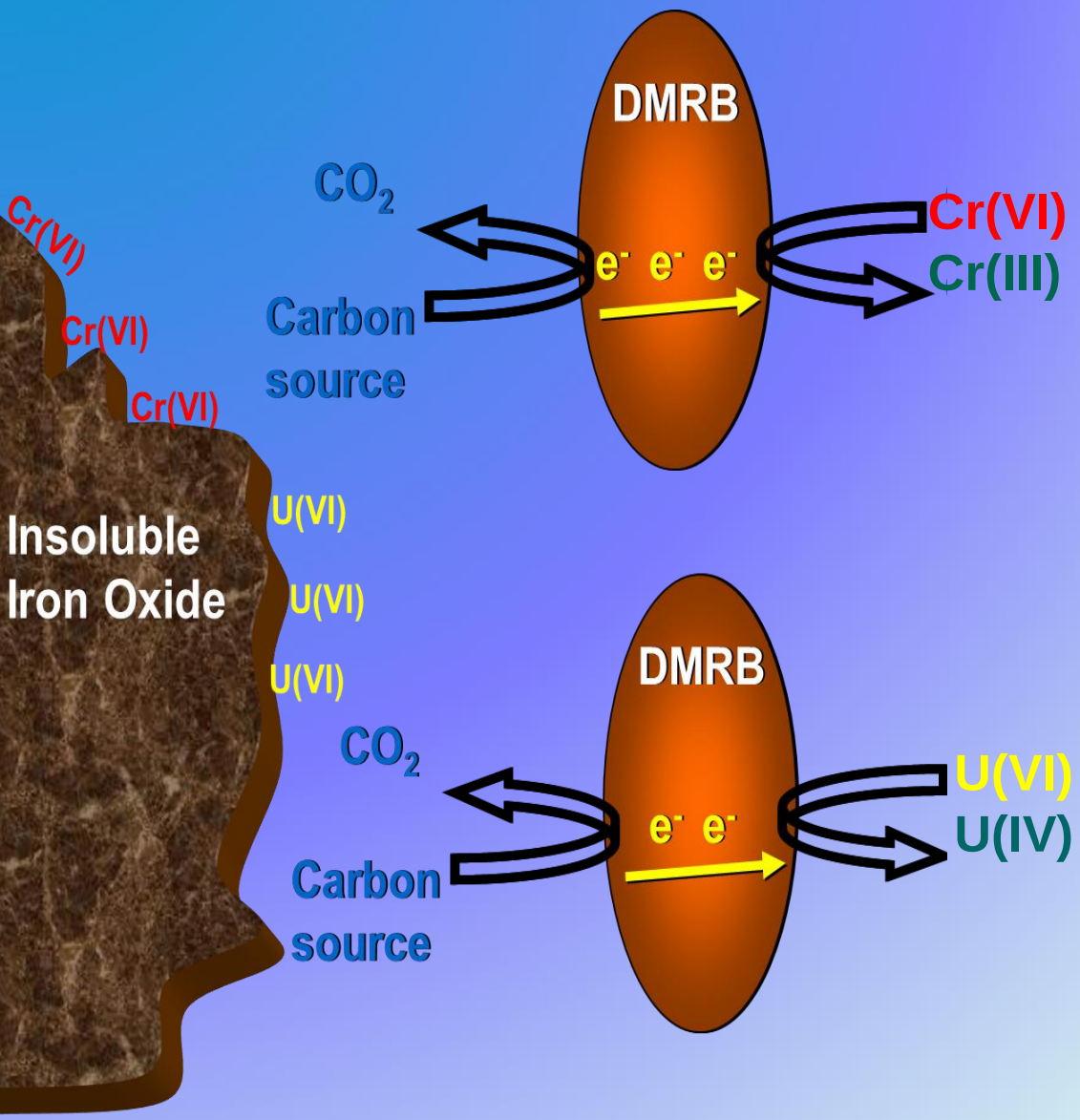
Bioremediation. J. 2:1-6

Next Challenge: Increase the rate of electron transfer to solid phase metal and actinide contaminants



Bacterial electron transfer to metal contaminants like Cr(VI) is impeded when the metals are sorbed to soil particles because the contaminants are part of the solid phase. This limits but does not negate their bioavailability. In order to increase bacterial electron transfer rates to solid oxidized metals and actinides we first had to drop back to more fundamental studies to understand the mechanisms of solid phase electron transfer.

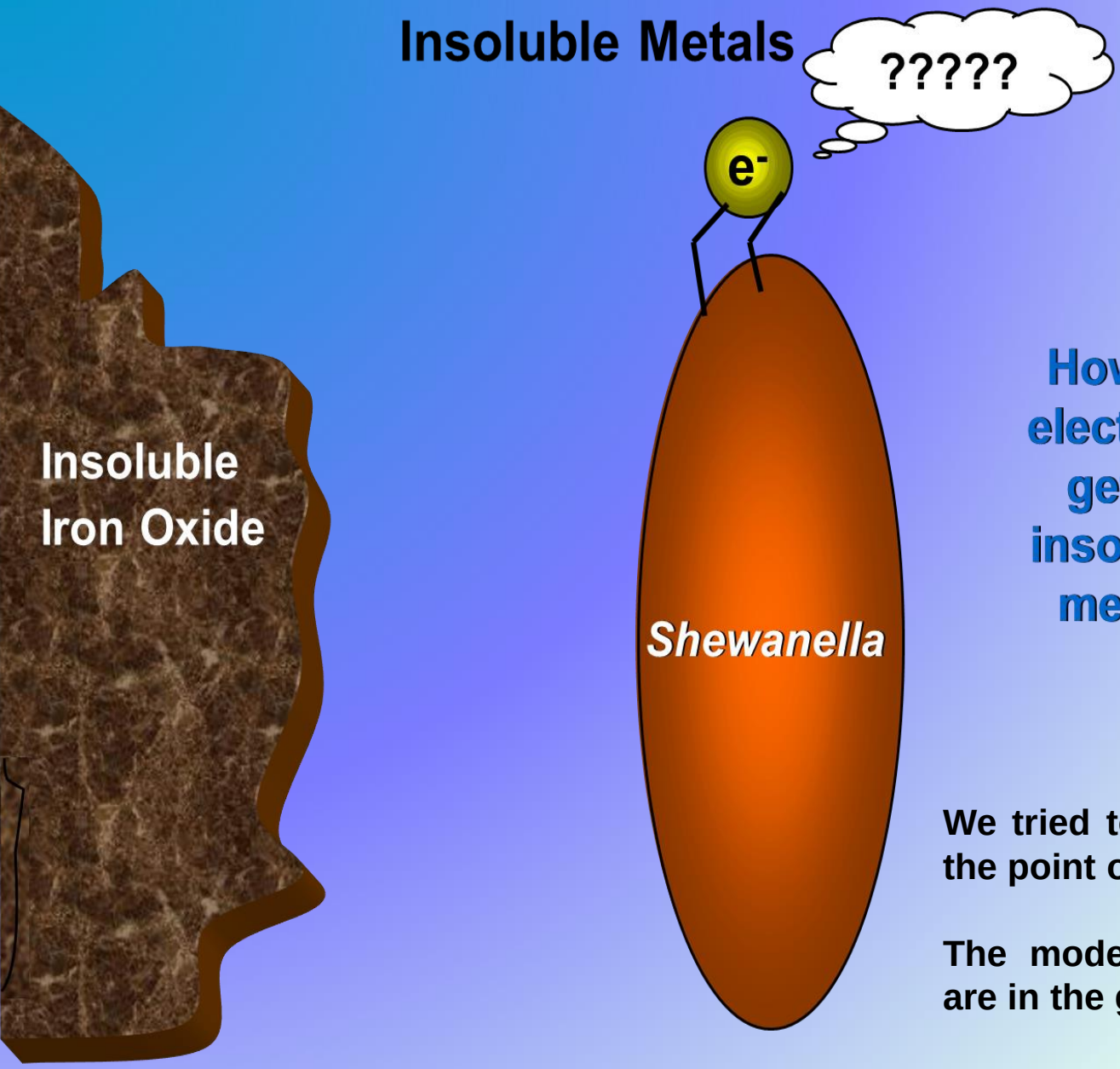
Soluble vs Solid Phase Metals



DMRB can use many metal oxides as terminal electron acceptors to respire when oxygen is absent. This is especially easy when the metals are in solution.

Transferring electrons from the bacterial cell outside to solid phase terminal electron acceptors requires some mechanism to send the electrons from the cell.

Understanding and exploiting mechanisms for extracellular electron transfer will increase the efficiency of bioremediation of heavy metals and radionuclides.

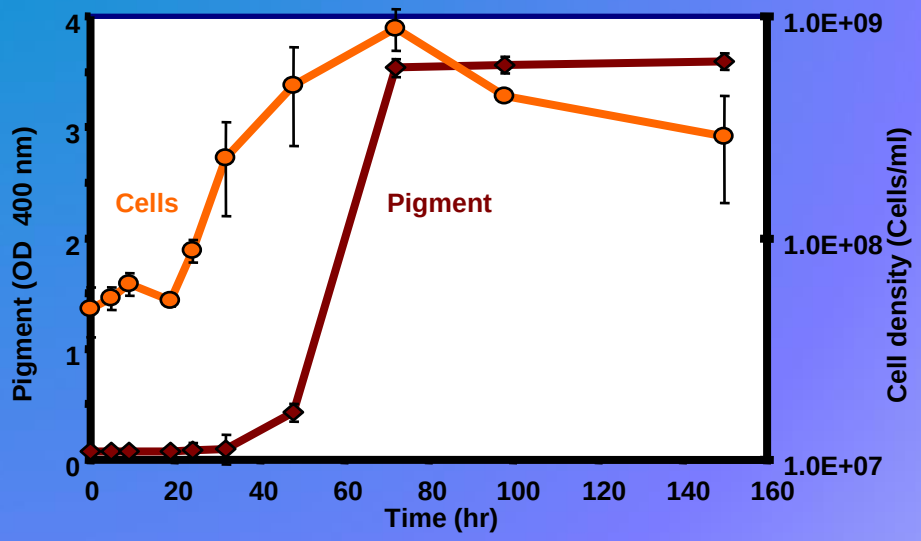


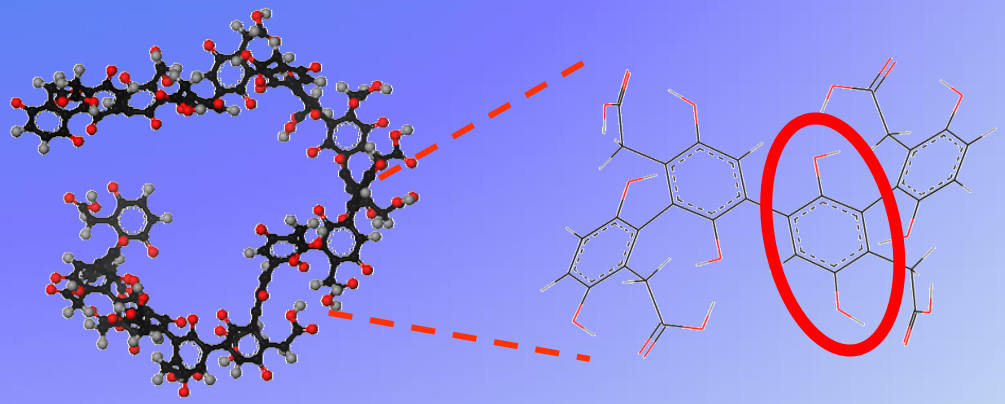
How do electrons get to insoluble metals?

We tried to see the problem from the point of view of an electron.

The model DMRB we work with are in the genus *Shewanella.*

Growth and Pigment Production





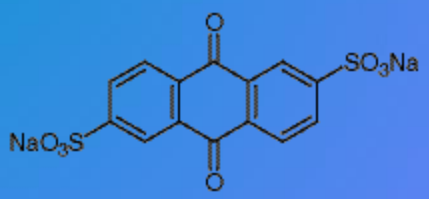
Many species of *Shewanella* produce the extracellular polymer pyomelanin from tyrosine degradation. The polymer is rich in the redox cycling structure – quinones.

This offered promise as an electron shuttle to bridge the gap between bacteria and solid phase metal oxides.

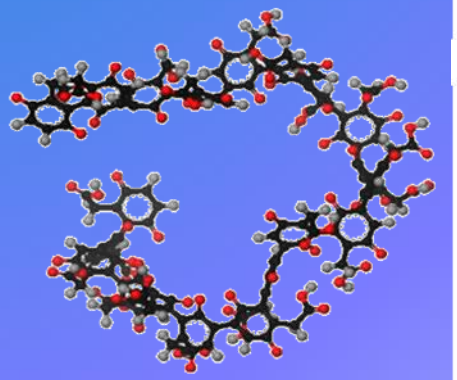
Applied Env. Microbiol. 68: 2436-2444

http://www.intechopen.com/books/show/title/biopolymers

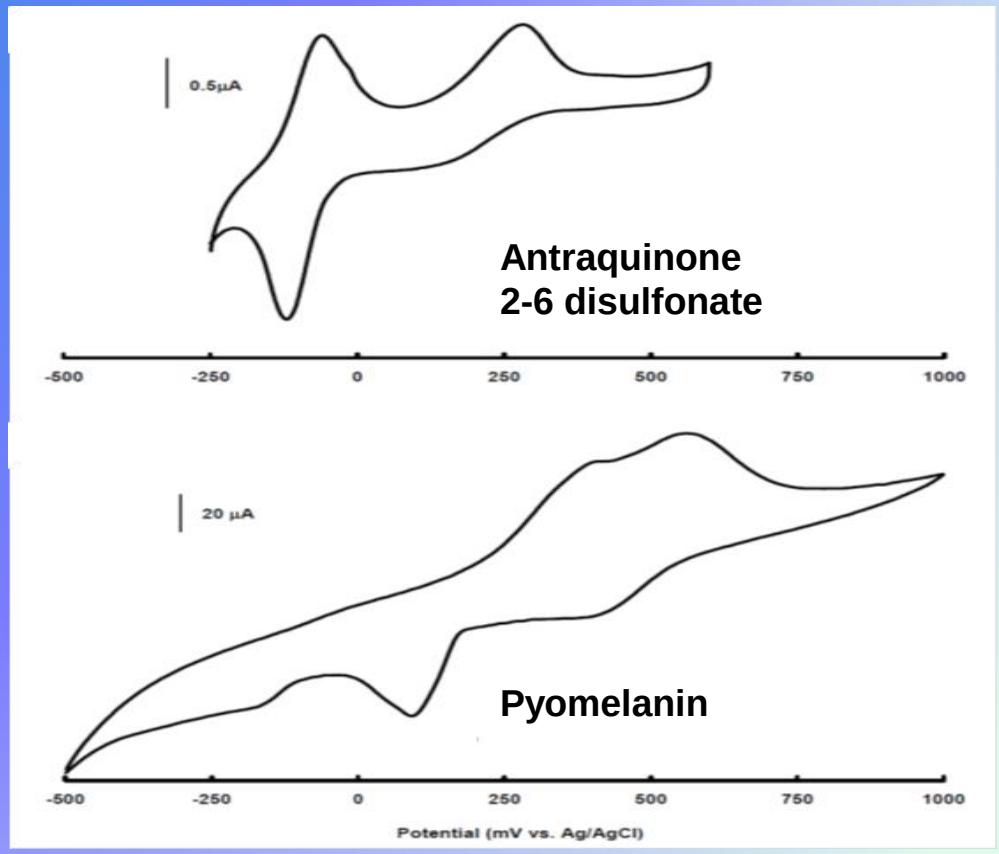
Electrochemistry of Pyomelanin



Antraquinone
2-6 disulfonate



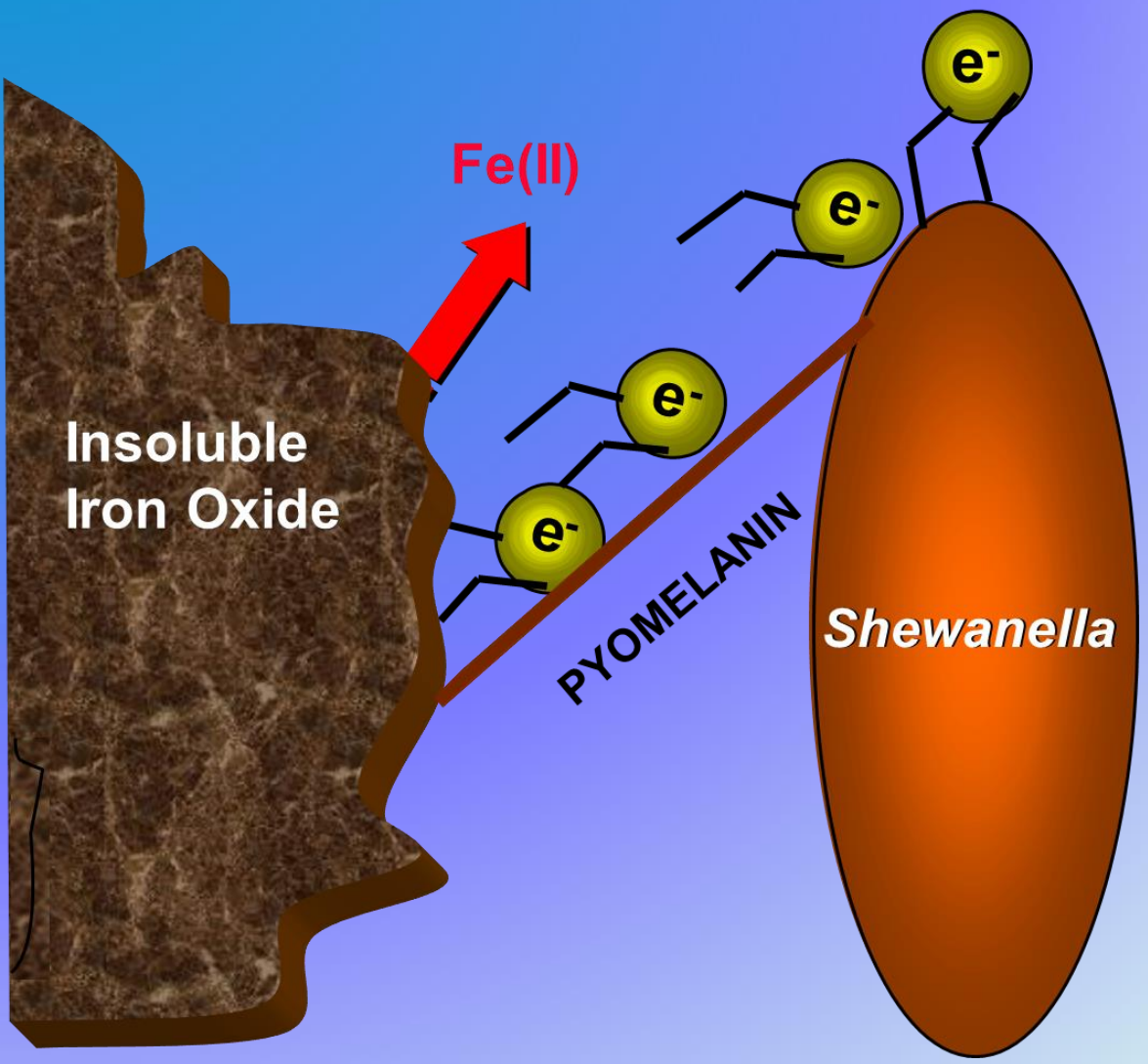
Pyomelanin



When evaluated with an electrochemical technique called cyclic voltammetry, pyomelanin demonstrated redox activity similar to another quinone containing molecule. With this technique the electrical potential (mV) is scanned from least to most oxidizing (left to right) and then least to most reducing (right to left). The two oxidation peaks (up) and 2 reduction peaks (down) are typical of quinones.

http://www.intechopen.com/books/show/title/biopolymers

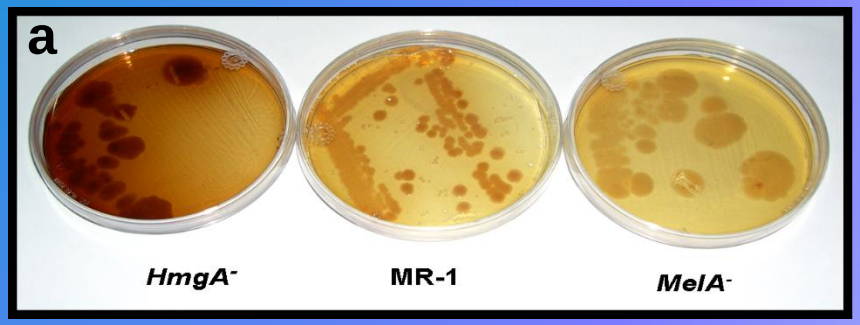
Pyomelanin Enhances Extracellular Electron Transfer

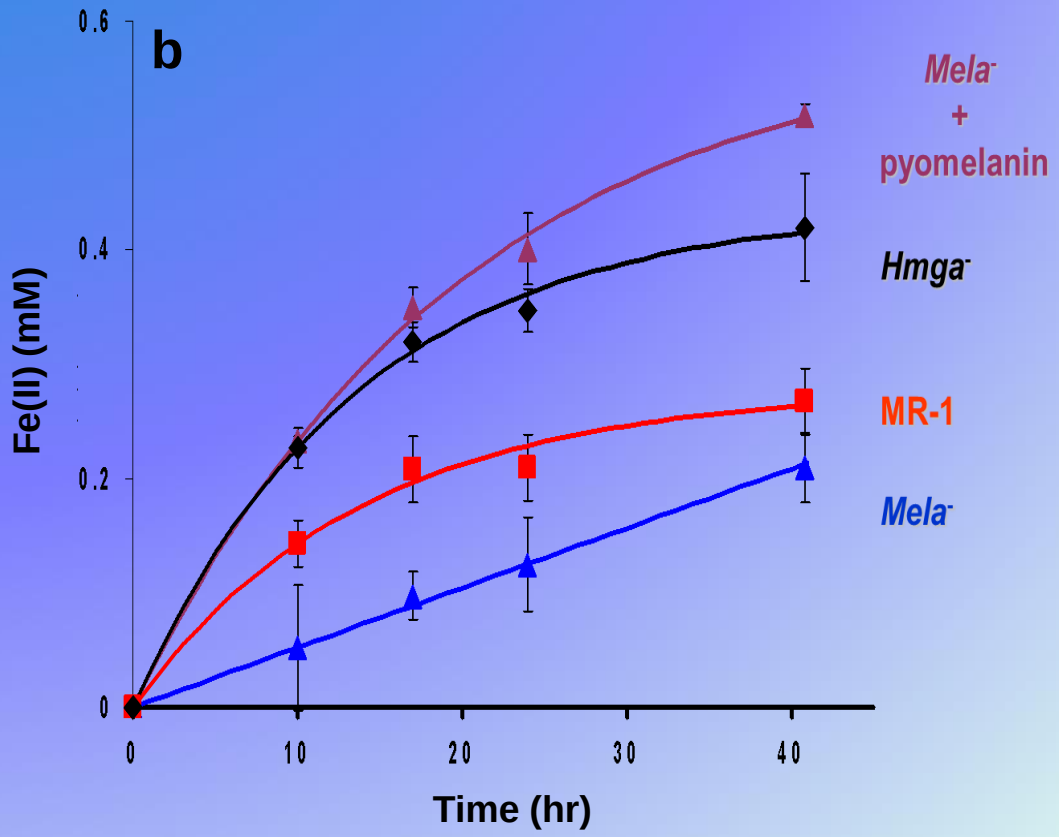


Pyomelanin produced by several strains and species of *Shewanella* enhance extracellular electron transfer to metal oxides.

FEMS Microbiol. Lett. 220:99-104
Can J. Microbiol. 54:334-339

Pyomelanin as an Electron Shuttle

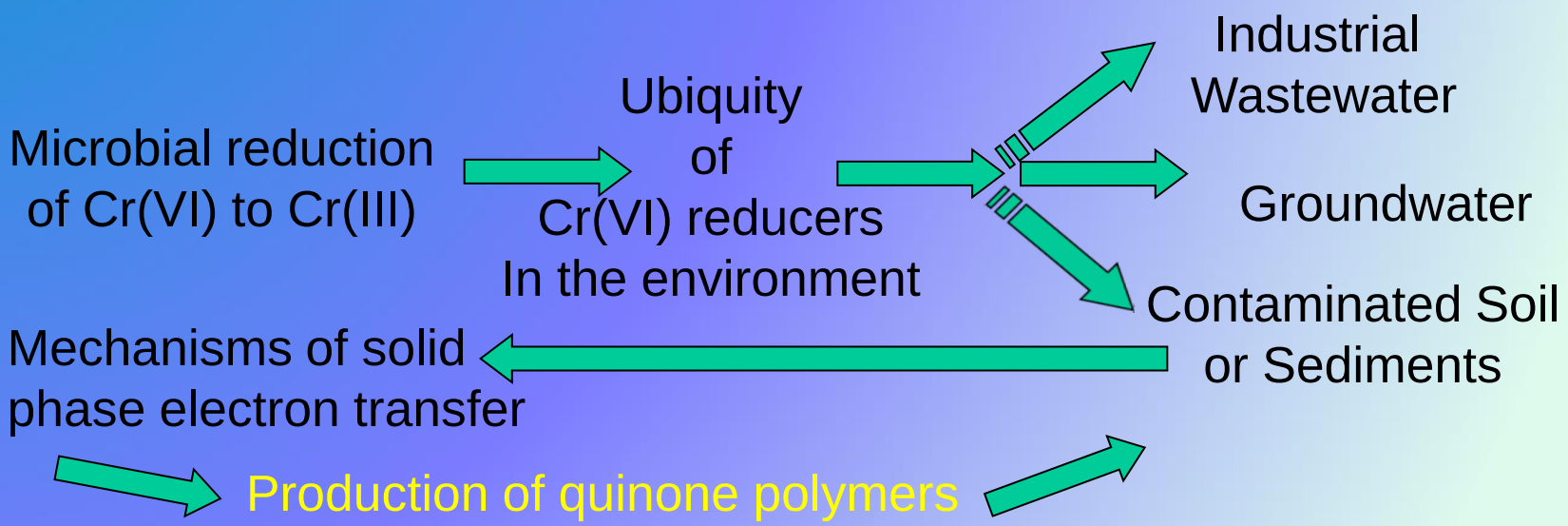




S. oneidensis **MR-1 along with mutants of that strain that included a pyomelanin over producer and a pyomelanin minus mutant (a) were used to show that pyomelanin plays an important role in enhancing extracellular electron transfer to solid phase metal oxides (in this case Fe(III) oxides) (b). The addition of soluble pyomelanin to the melanin minus mutant also increased its rate and degree of metal reduction.**

FEMS Microbiol. Ecol. 68:223-235
Appl. Environ. Microbiol. 68:2436–2444

Next Challenge: Increase the rate of electron transfer to solid phase metal and actinide contaminants



The production of electroactive polymers by some bacteria bridge the gap for electron transfer to metal oxides. At least in the lab.
Next try: enhance electron transfer in the environment.

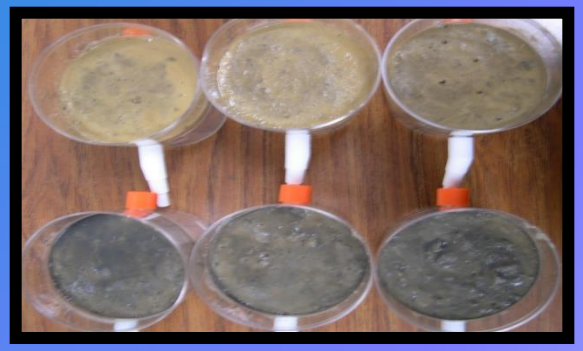
Pigment Producing Microbes in Soil

Soil Assay

a

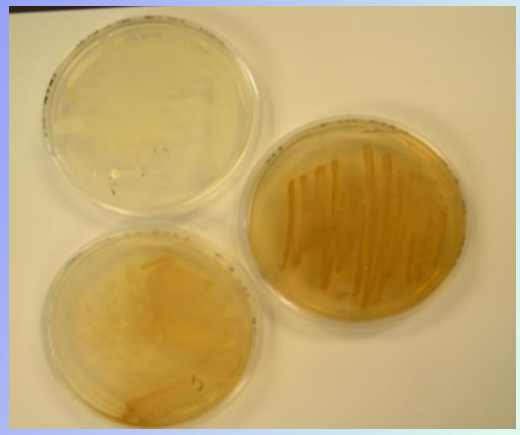
Control

Tyrosine



We were able to stimulate production of a dark pigment in soil after addition of tyrosine (a). Bacteria capable of pyomelanin production (b) were the most common pigment producers in the soils we were studying.

b



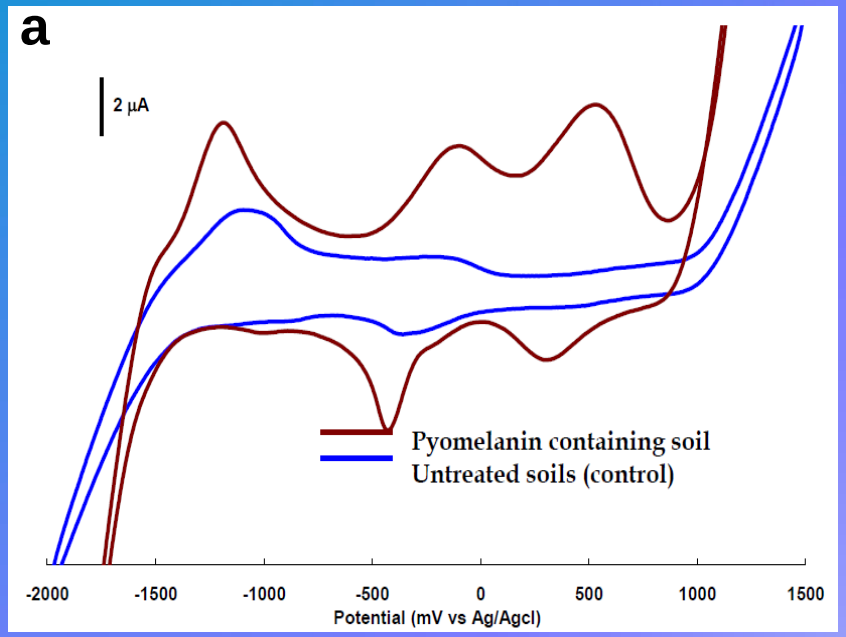
Most common pigment producer tentatively identified as *Bacillus mycoides*

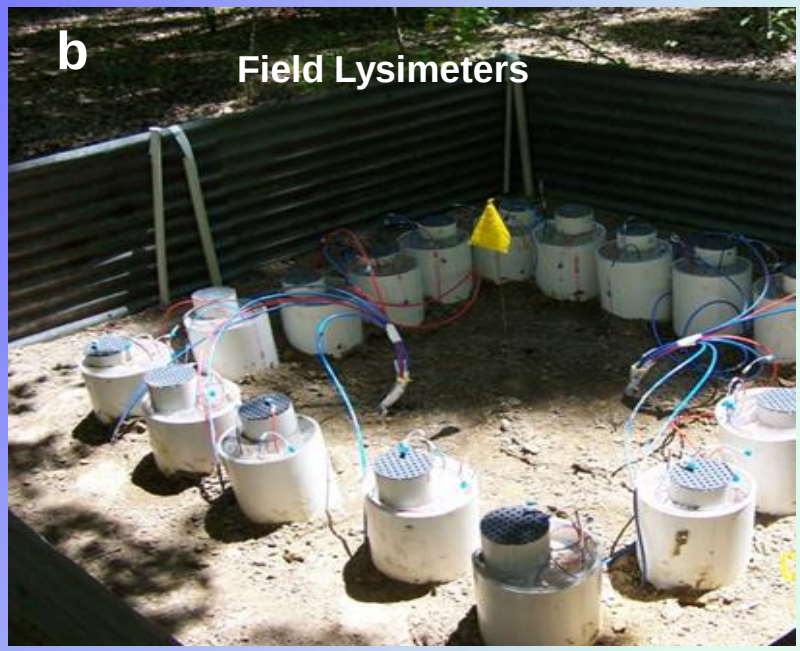
Pigment produced was characterized as pyomelanin

MPN results

1.1×10^6 cells/g wet wt of soil

Pigment Producing Microbes in Soil



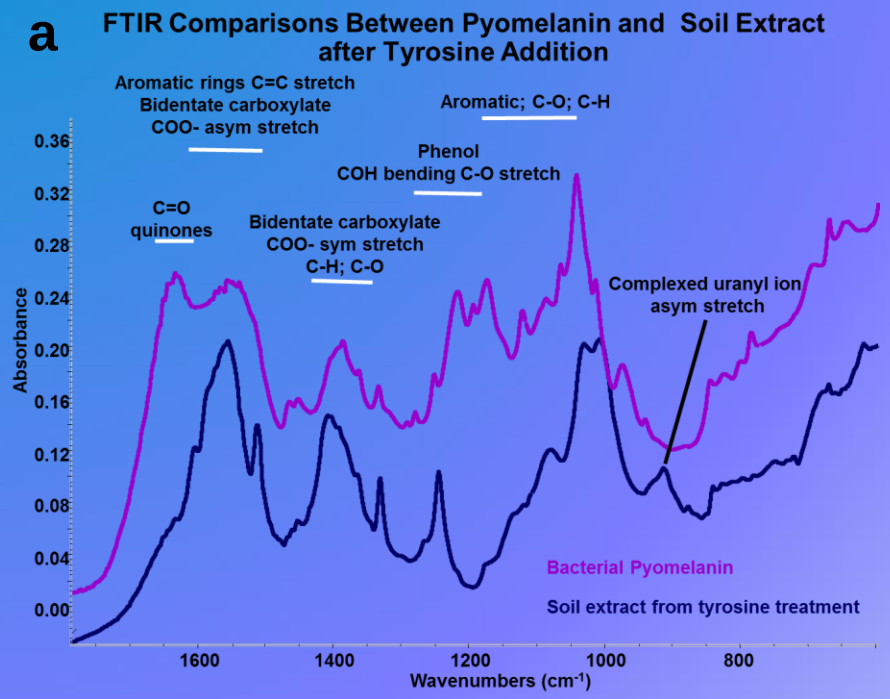


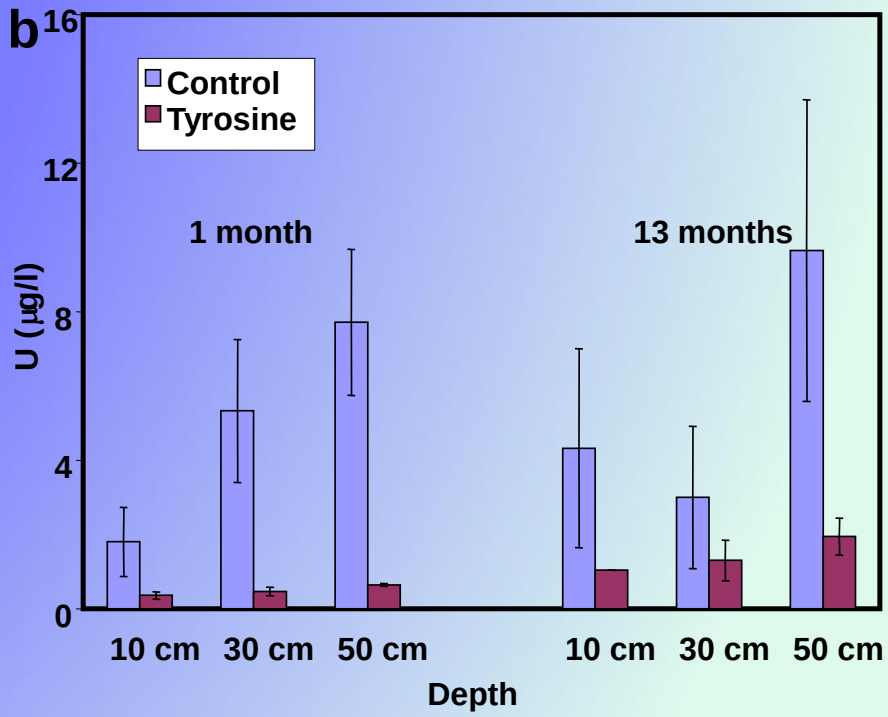
Soil with the pyomelanin pigment was much more electroactive compared to the untreated soil. Electrochemical studies showed 2 oxidation peaks (upward) and 2 reduction peaks (downward) between -1 and 1 volt (a). This behaves as we expect quinone containing polymers and shows that we were able to change the electrochemistry of the soil. The increase in electron transfer suggests that with pyomelanin, soluble and mobile U(VI) contaminants could be reduced and immobilized in the soil. So we set up an experiment in U(VI) contaminated soils to try to immobilize U in place (b).

J. Environ. Radioact. 99:890-899

http://www.intechopen.com/books/show/title/biopolymers

Melanin Effects on U Immobilization

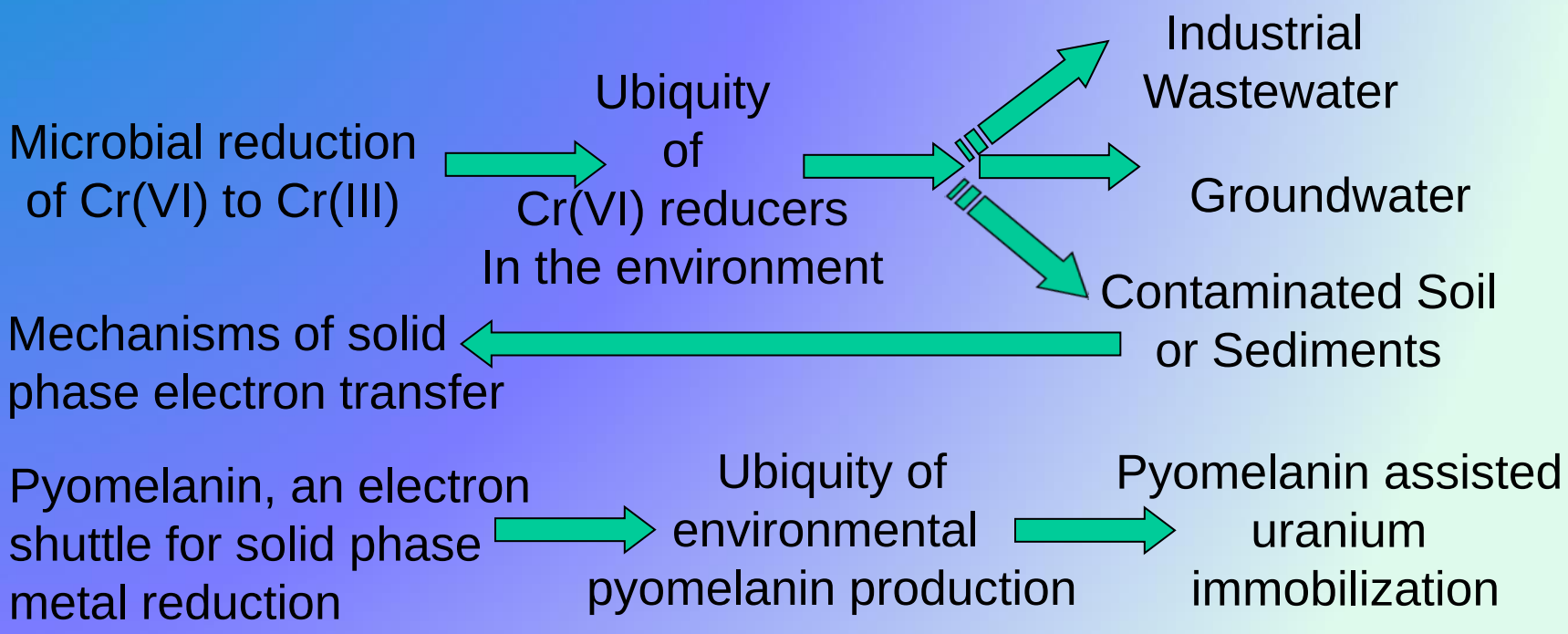




The soil pigment was compared to bacterial pyomelanin and showed many similarities (a). Differences were likely do to OH and COOH groups binding uranium and also attaching to soil particles. Because of that, the pigment was able to reduce U(VI) and also "tether" it to soil particles resulting in immobilized uranium (b). Just one small application of tyrosine resulted in pyomelanin production and uranium immobilization that lasted over one year.

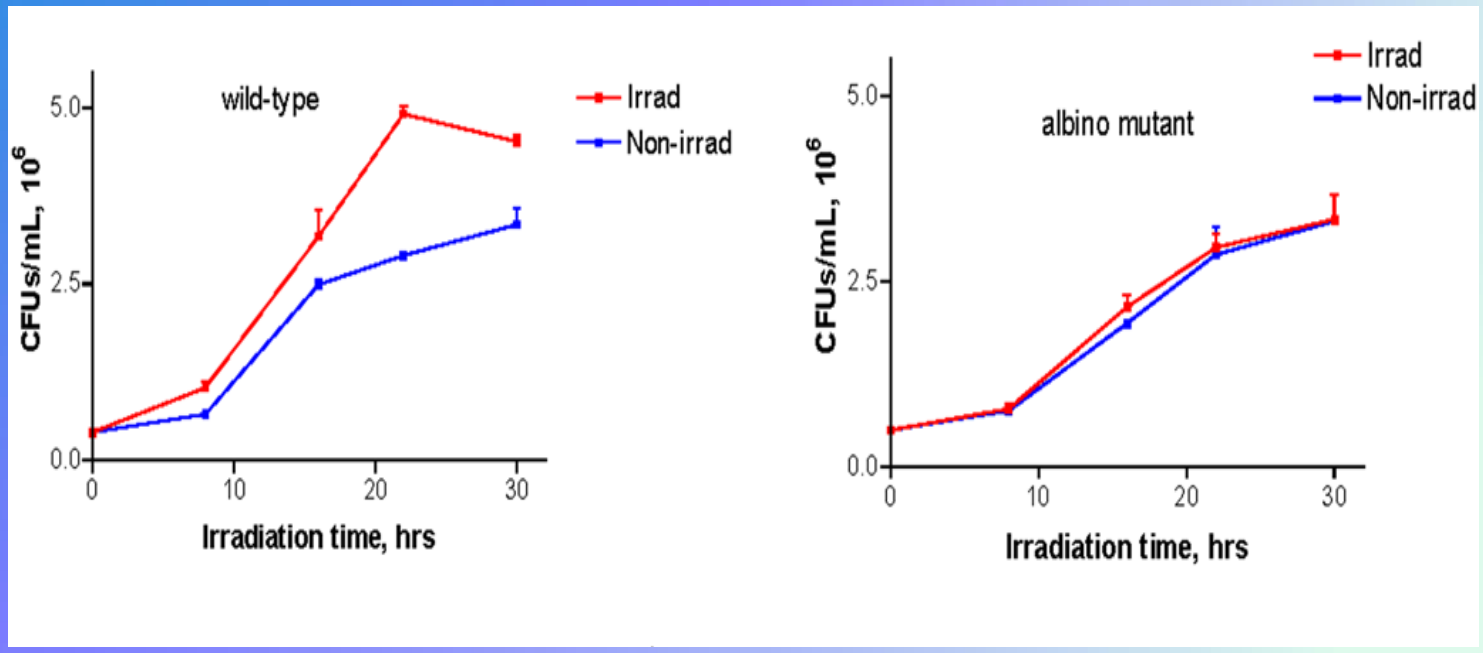
J. Environ. Radioact. 99:890-899

Challenge met: Increased the rate of electron transfer to solid phase metal and actinide contaminants.



By controlling microbial production of electron shuttles in the soil we were able to significantly enhance electron transfer to contaminants in the environment, leading to contaminant immobilization.

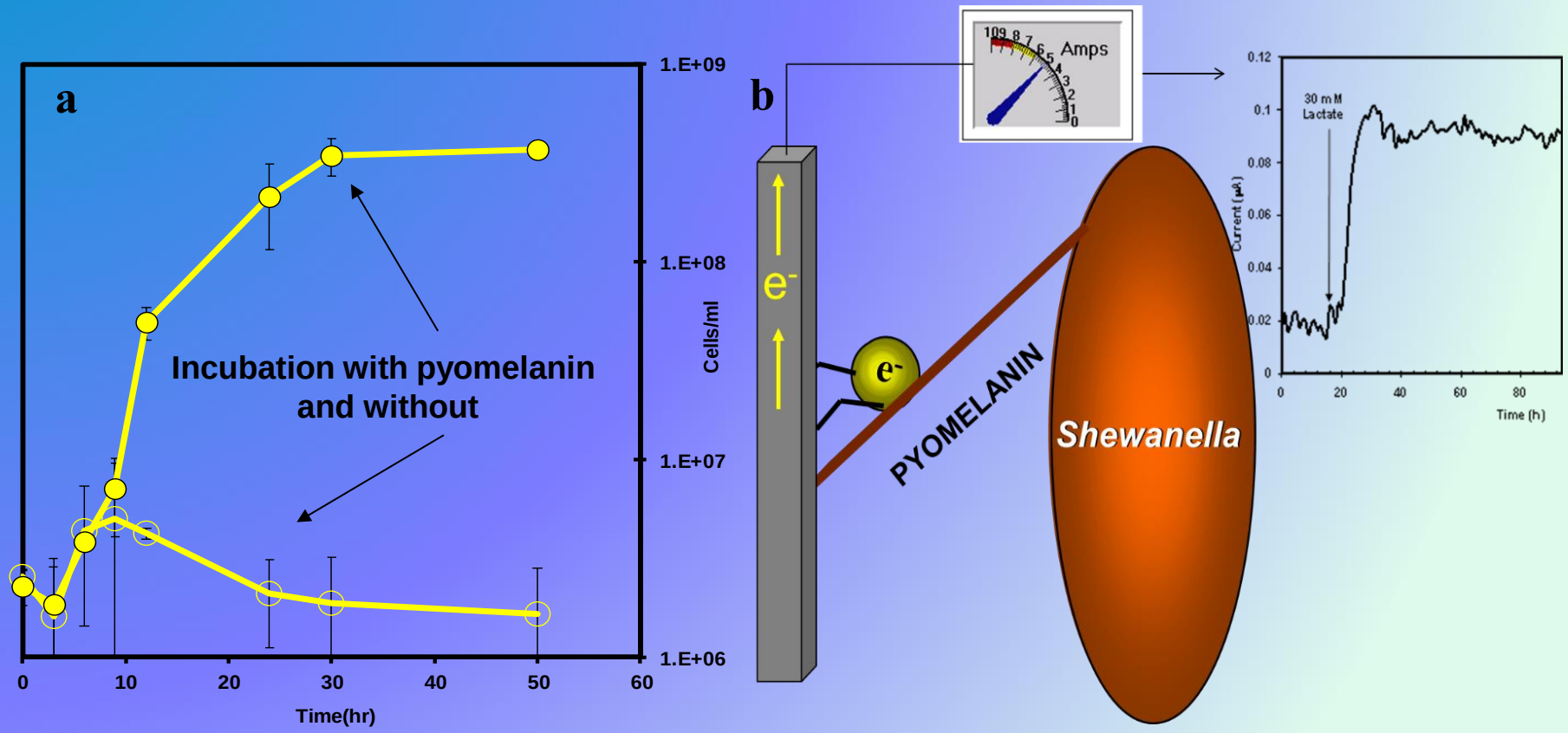
New Challenge: How do dark-pigment-producing fungi that are exposed to chronic, high levels of gamma radiation (i.e. Chernobyl reactor facility) actually grow better in the presence of radiation?



Growth of *Wangiella dermatitidis* with/without γ irradiation (~500x background)

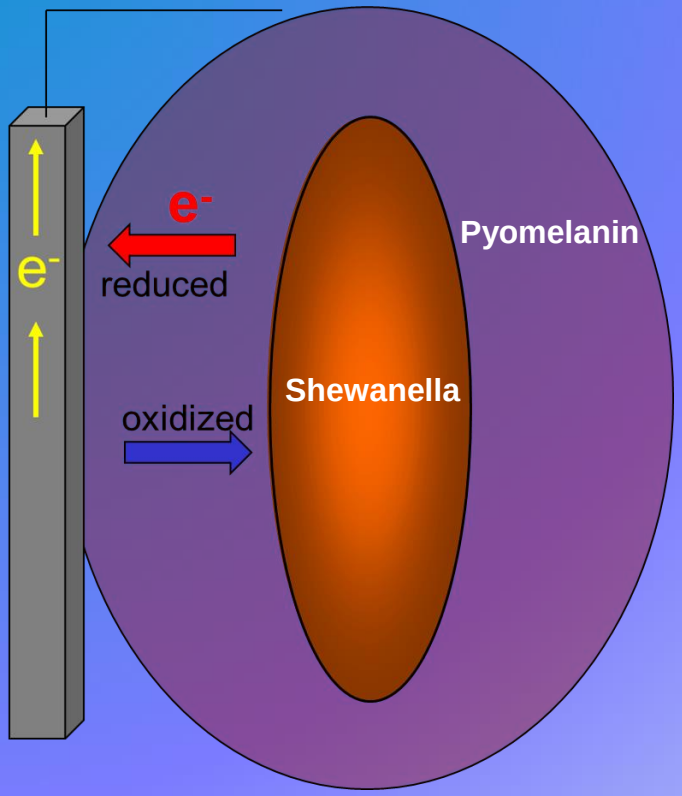
PLoS ONE. 5:e457

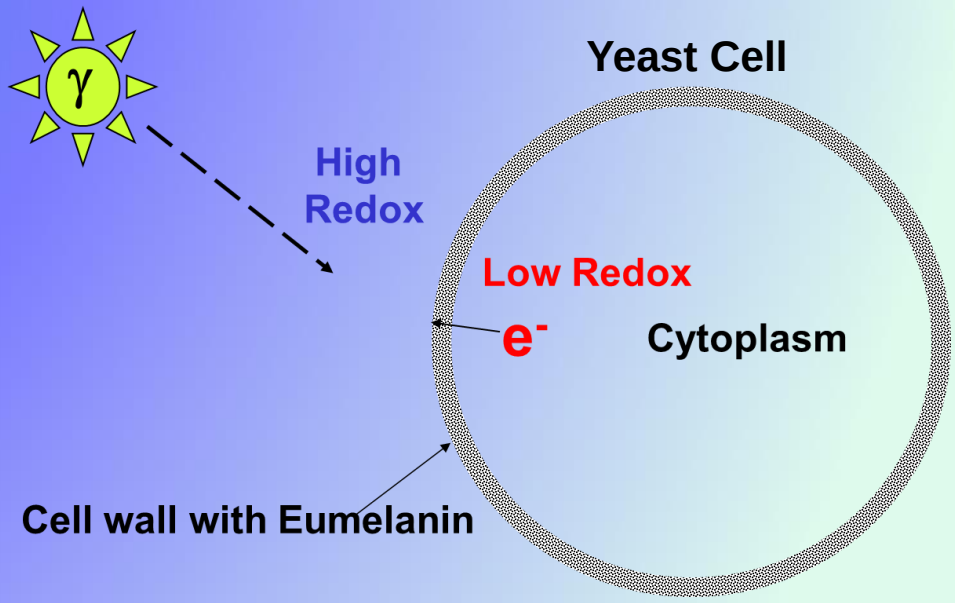
Clue: *Shewanella* can use the pyomelanin they make as a terminal electron acceptor when O_2 is absent.



The bacteria could transfer electrons to oxidized pyomelanin and grow (a). When we included an electrode, pyomelanin acted as an electron conduit so the electron flow could be monitored with electrochemical techniques (b). This led to an idea about how some microbes might grow better with ionizing radiation and how we could study them.

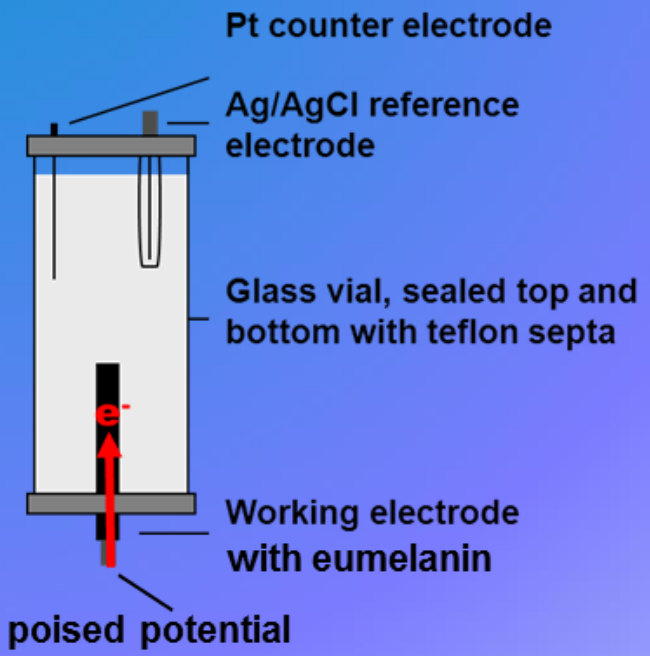
Electron Transfer with Extracellular Melanin





The fungi that grow well in radiation fields all produce the pigment eumelanin, a similar pigment to pyomelanin. A constantly oxidized electrode takes electrons from reduced pyomelanin, restoring the pyomelanin back to the oxidized state. Could gamma radiation constantly oxidize fungal melanin and act as a "bottomless pit" for electrons?

Gamma Exposure ($4x10^5$ rad/hr) to Various Concentrations of Eumelanin



In order to test the hypothesis that radiation turns eumelanin into a "bottomless pit" for electrons we set up the following experiment. With eumelanin isolated from the surface of fungal cells we constructed an electrode and placed it next to a radiation source. With the electrodes connected to a potentiostat, a potential was applied to the eumelanin electrode. Next we turned on the radiation. If gamma radiation oxidized the eumelanin an electric current would flow.

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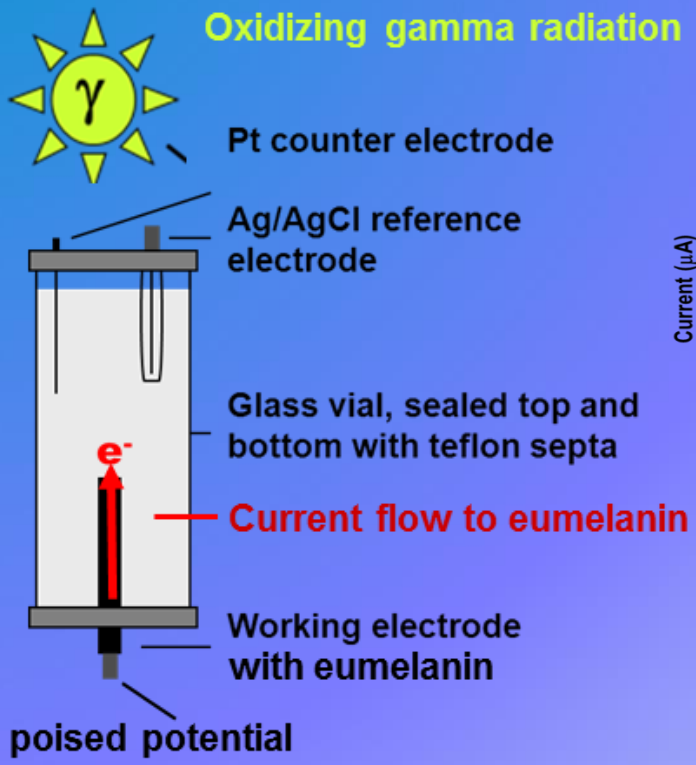
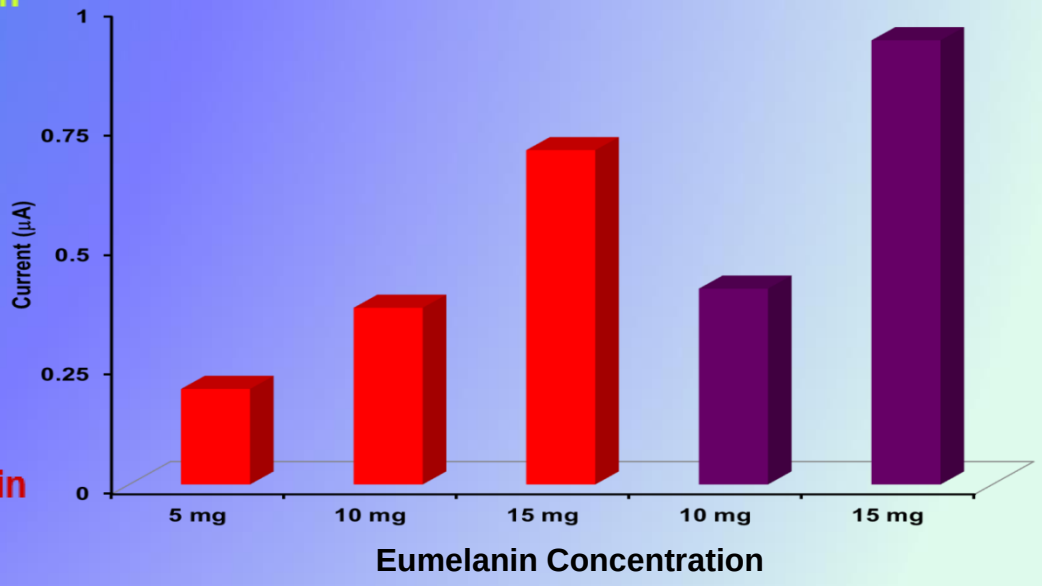
Gamma Exposure ($4x10^5$ rad/hr) to Various Concentrations of Eumelanin





60 min. exposure

90 min. exposure

Irradiated eumelanin was able to allow electrons to flow through it. The more eumelanin in the electrodes and the longer the exposure time, the more current was produced.

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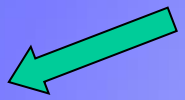
Electron transfer to gamma irradiated eumelanin

How do microorganisms thrive in radioactive environments

Irradiated eumelanin Is a bottomless pit for electrons

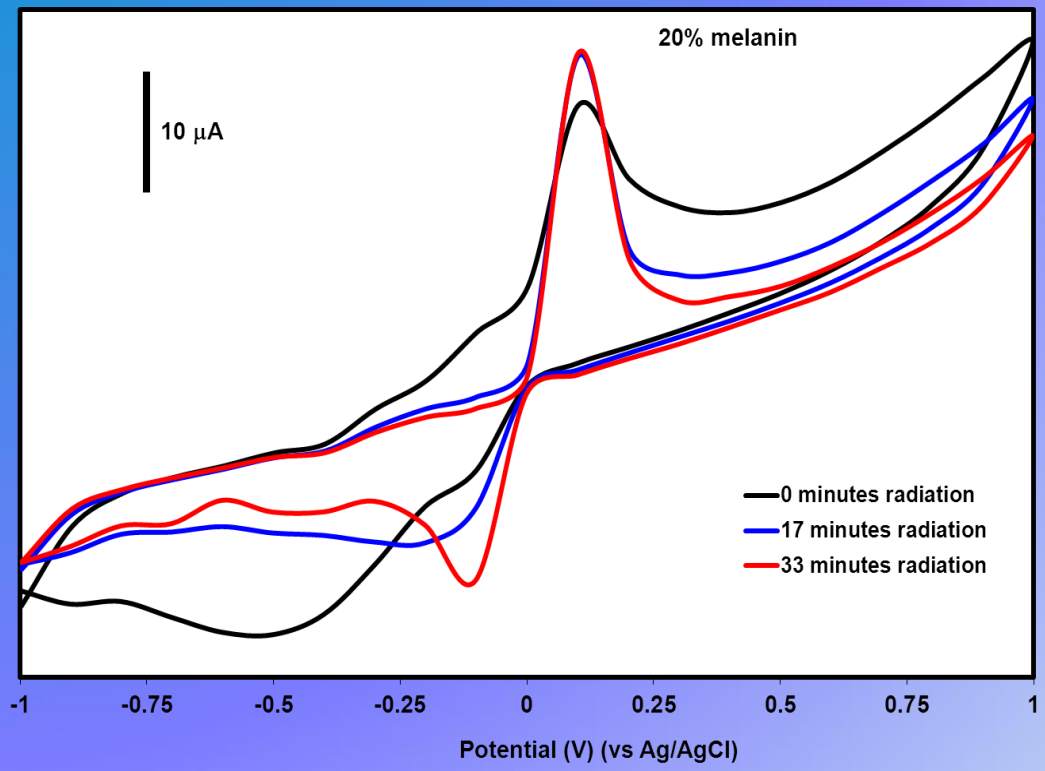


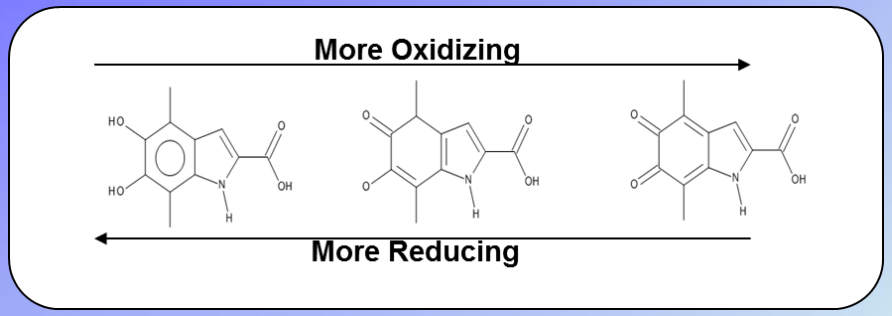
Why doesn't the eumelanin get bleached by all the radiation?

A plausible answer to one question raised another interesting question. The tremendous oxidizing power of the radiation we used was enough to oxidize the eumelanin. The chronic levels of radiation encountered by the microbes in the Chernobyl nuclear facility should also oxidize them.

Why aren't they all bleach blondes?

A Mechanism of Radiation Protection





Cyclic voltammetry of the eumelanin electrodes showed that the polymer is oxidized by radiation (upward pointing peaks). The addition of electrons restore the chemical structure of eumelanin to a reduced state (downward pointing peaks).

This could be a radiation protection mechanism that also allows some microbes to gain energy at the same time.

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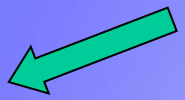
Challenge met: Electron transfer to gamma irradiated eumelanin

How do microorganisms thrive in radioactive environments

Irradiated eumelanin Is a bottomless pit for electrons



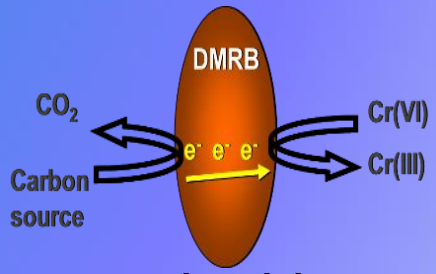
Why doesn't the eumelanin get bleached by all the radiation?

Continuous electron transfer restores oxidized eumelanin.

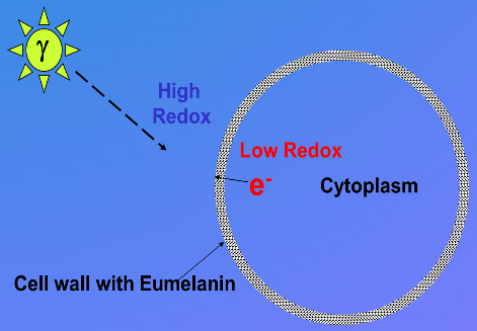
As a bottom-less pit for electrons, fungal eumelanin is also chemically restored as a radiation protecting molecule.

Conclusions

Our fundamental studies are moving to applied science and technology developments as summed up below.



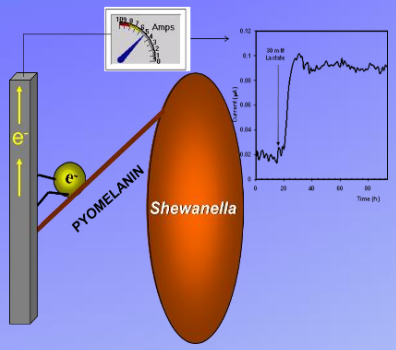
Industrial biotechnology



Bio-inspired materials

Fundamental studies in microbial electron transfer are leading to:

Enhanced solid-phase electron transfer



Electromicrobiology

In-situ bioremediation

Acknowledgements

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