

SILVANALA

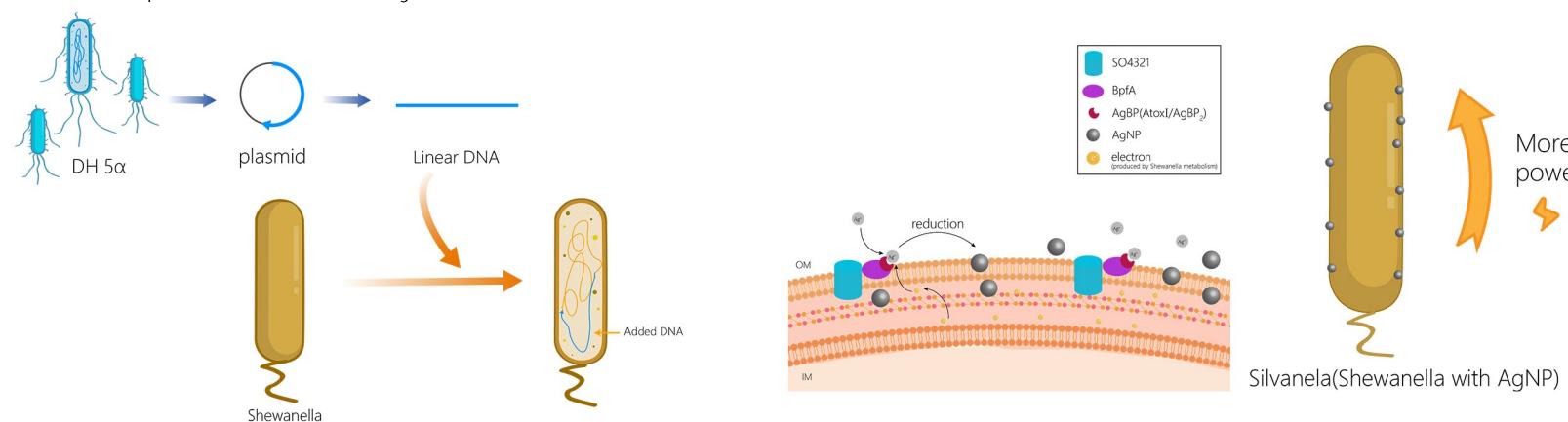


NANTING CHINA
iGEM 2022

We constructed engineered Shewanella using silver ion-binding peptide (AgBP). The fuel cells were then constructed using the engineered Shewanella bacteria. Sustained Shewanella culture solution was inserted into the anode to provide a constant voltage, and ferric thiocyanate was inserted into the cathode. The anode is in an anaerobic environment at this time. In order to get close to the anode, which is the only acceptor of electrons produced by biological oxidation, Shewanella will spontaneously accumulate on the anode to form a biofilm with a considerable thickness. During this process, a low concentration of silver nitrate was added to the culture solution. We designed a short silver-binding peptide on the C-terminus of the membrane protein to capture silver ions. During the biooxidative electron transfer process, silver ions are reduced and aggregated into silver nanoparticles. The silver short peptides on the membrane protein played the role of enriching silver ions, so that the silver

Working Principle

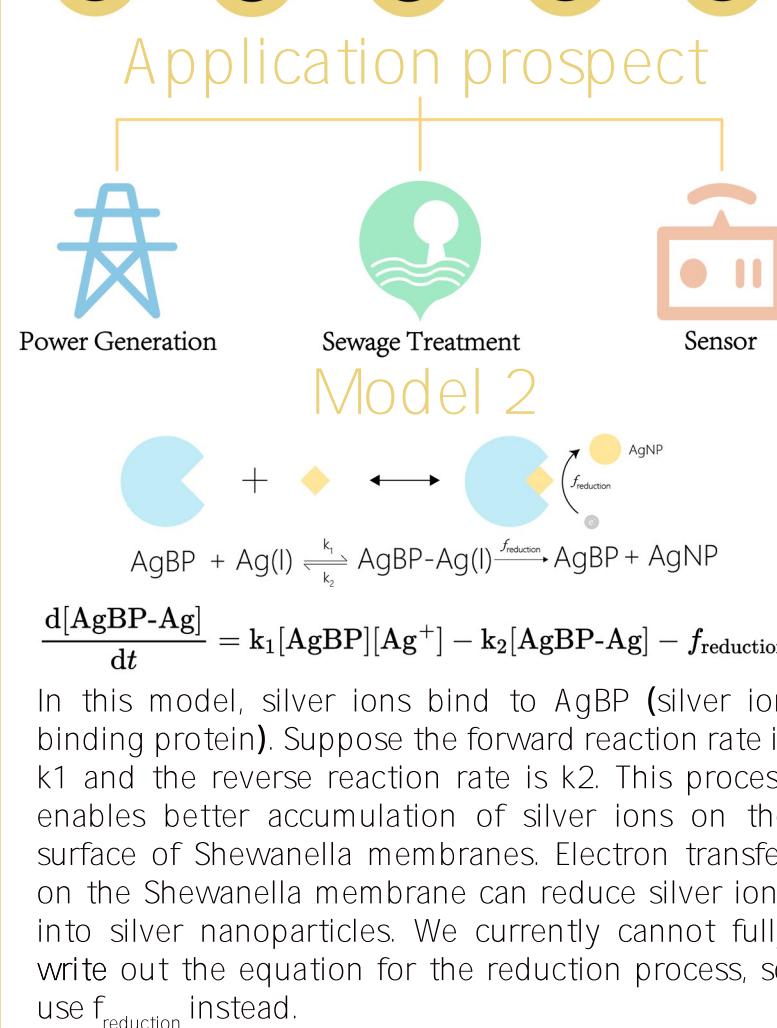
nanoparticles (AgNPs) were more concentrated in the outer membrane and periplasmic space. Considering that silver is much more conductive than cytochromes, this approach may help speed up the process of electron transfer and improve cell efficiency.



POSITIVE

Background

The current era can be called the "world energy era", and the hot issues of the world energy situation have become a global issue that has attracted worldwide attention. At present, the world's energy use structure is still dominated by the three traditional energy sources of oil, natural gas and coal, supplemented by clean energy such as nuclear energy, wind energy and biomass energy. However, fossil energy reserves are no longer optimistic. It can be seen that the research and development of new energy is urgent. Microbial fuel cells provide us with certain ideas in terms of electrical energy. The concept of using microorganisms as catalysts in batteries has been proposed as early as the 1970s, and the concept of microbial fuel cells has been continuously improved since then. Today's microbial fuel cells mainly use microorganisms attached to the anode as catalysts to degrade organic matter, generate electrons in the process of decomposing organic matter, and transfer them to the anode through an external circuit to reach the cathode, thereby generating an external current. Microbial fuel cells have the advantages of low cost, cleanliness and environmental protection, and high energy conversion efficiency. In addition to production capacity, they have more significant advantages in the field of environmental protection, and are widely used in the treatment of environmental pollution.



In this model, silver ions bind to AgBP (silver ion binding protein). Suppose the forward reaction rate is k_1 and the reverse reaction rate is k_2 . This process enables better accumulation of silver ions on the surface of Shewanella membranes. Electron transfer on the Shewanella membrane can reduce silver ions into silver nanoparticles. We currently cannot fully write out the equation for the reduction process, so use $f_{\text{reduction}}$ instead.

NEGATIVE

Model 1

$$\begin{cases} \frac{dn_a}{dt} = +\beta n_s + k_0 \left(1 - \frac{n_a}{E - n_d - n_s} \right) n_a \\ \frac{dn_s}{dt} = -\alpha n_s - \beta n_s \\ \frac{dn_d}{dt} = +\alpha n_s \end{cases}$$

We established the model shown in the figure with reference to Haque et al.'s study on the effect of silver ions on E. coli growth. It can be used to evaluate the effect of silver ions on the growth of Shewanella. In this model, bacteria affected by silver ions are in the Suppressed(s) state. Bacteria in the S state have the potential to enter the Dead(d) state. Let the mortality rate in this process be α . It is also possible for bacteria in the S state to enter the Active(a) state. Suppose the activation rate in this process is β . Bacteria in state a are able to reproduce continuously. Suppose their growth rate is k_0 . However, their growth is limited by E (asymptotic cell number) due to the limitation of the culture environment. This model can help us explore the suitable silver ion range for Shewanella to survive.

Construction of expression vector

The pUC57-BpfA-Atox1/AgBP2-Kana-loxP-agc fusion vector contains a core DNA box consisting of the coding sequence of a silver binding protein (either Atox1 and AgBP2, hereinafter referred to as AgBP) and the kanamycin resistance gene (Kana). The core DNA box enables BpfA to bind silver ions and helps us screen the recombinants. The upstream and downstream of our core DNA box are two sequence fragments of the MR-1 homologous gene BpfA (3' end) and aggC (5' end), both of which are 1000 bp in length, used to realize the recombination transformation of MR-1 and the localization of silver-binding protein. The entire design is based on the pUC57 (mini) vector, which carries the ampicillase resistance gene (AmpR).

S. oneidensis has no apparent codon usage bias, so genetic fusion of AgBP and BpfA can be performed without much codon optimization. To create the AgBP knock-in construct, using the wild-type S. oneidensis MR-1 genome as models, the homology arm sequences were amplified using BpfA-F/R and AggC-F/R as primers, each 1000 bp in size. The AgBP sequence was synthesized by Qingke Biotechnology, and the Kana resistance gene fragment was amplified from the prokaryotic expression vector pET28a by the

primer Kana-F/R. After PCR amplification to obtain BpfA, AggC, Atox1, KanR sequences, the sequence size was verified through agarose gel electrophoresis, and the fragments were recovered and inserted into pUC57mini vector to construct pUC57mini-Atox1-Kana-loxP-AggC. To fuse Atox1 in-frame with the C-terminus of BPFA, the vector pUC57mini-Atox1-Kana-loxP-AggC was double digested with EcoRI and KpnI, and the vector-free linear fragment was purified for subsequent electroporation into MR-1.

