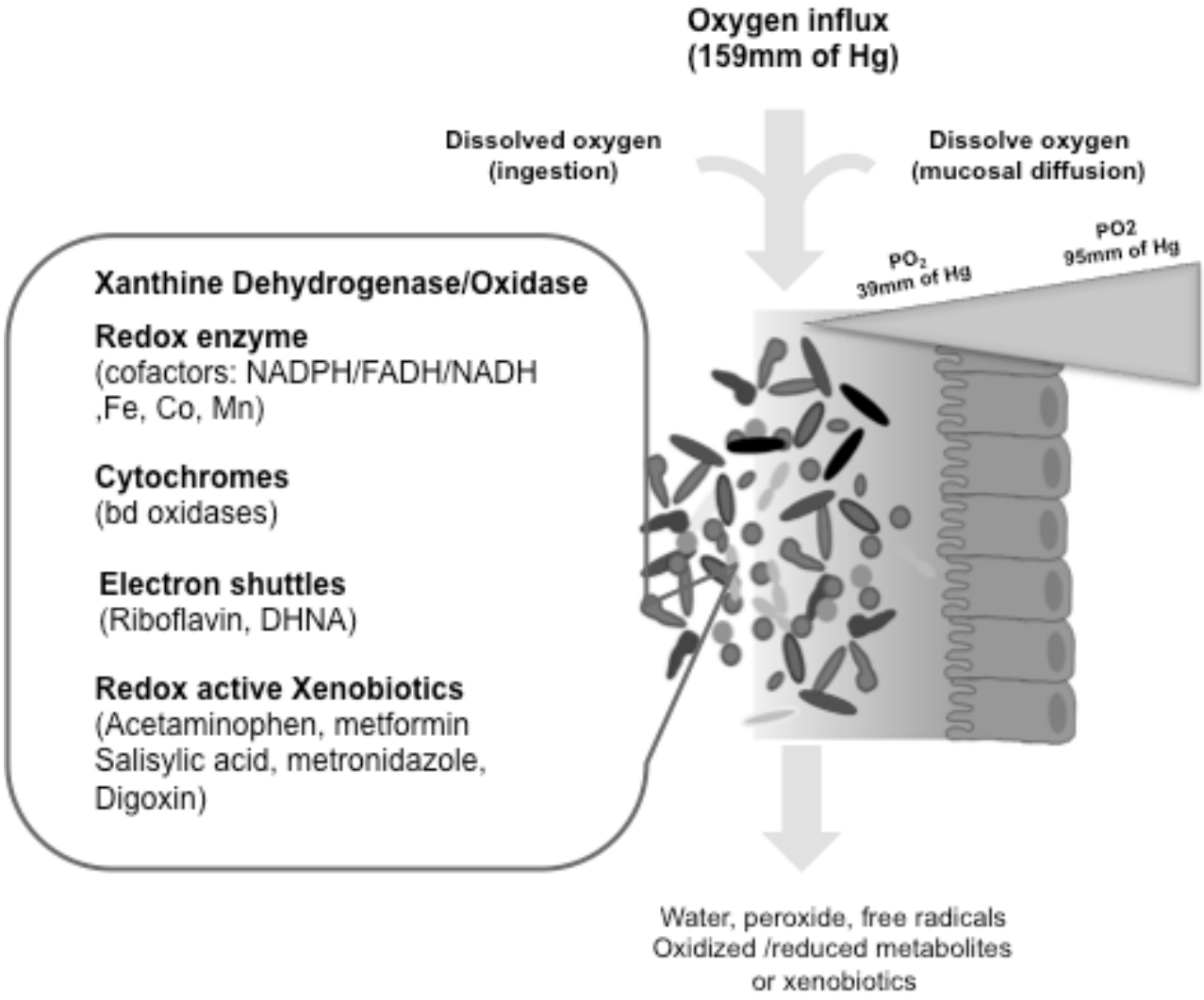

Oxygen influx
(159mm of Hg)
Dissolved oxygen
(ingestion)
Dissolve oxygen
(mucosal diffusion)
PO2
95mm of Hg
PO2
39mm of Hg
Xanthine Dehydrogenase/Oxidase
Redox enzyme
(cofactors: NADPH/FADH/NADH
,Fe, Co, Mn)
Cytochromes
(bd oxidases)
Electron shuttles
(Riboflavin, DHNA)
Redox active Xenobiotics
(Acetaminophen, metformin
Salisylic acid, metronidazole,
Digoxin)
Water, peroxide, free radicals
Oxidized /reduced metabolites
or xenobiotics

Figure 1

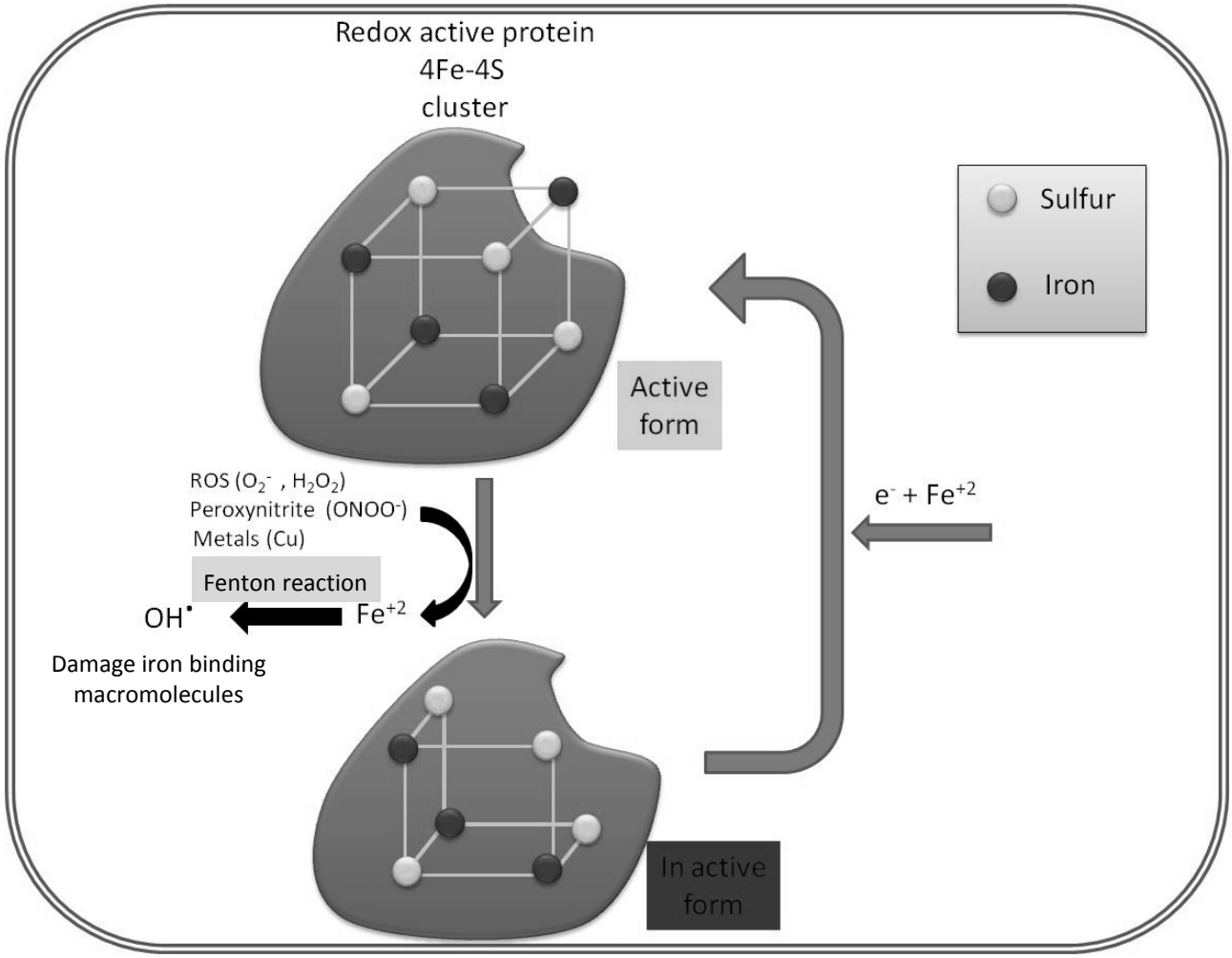

Redox active protein
4Fe-4S
cluster
Sulfur
Iron
Active
form
ROS (O_2^- , H_2O_2)
Peroxynitrite ($ONOO^-$)
Metals (Cu)
Fenton reaction
$OH^•$
Fe^{+2}
e^- + Fe^{+2}
Damage iron binding
macromolecules
In active
form

Figure 2

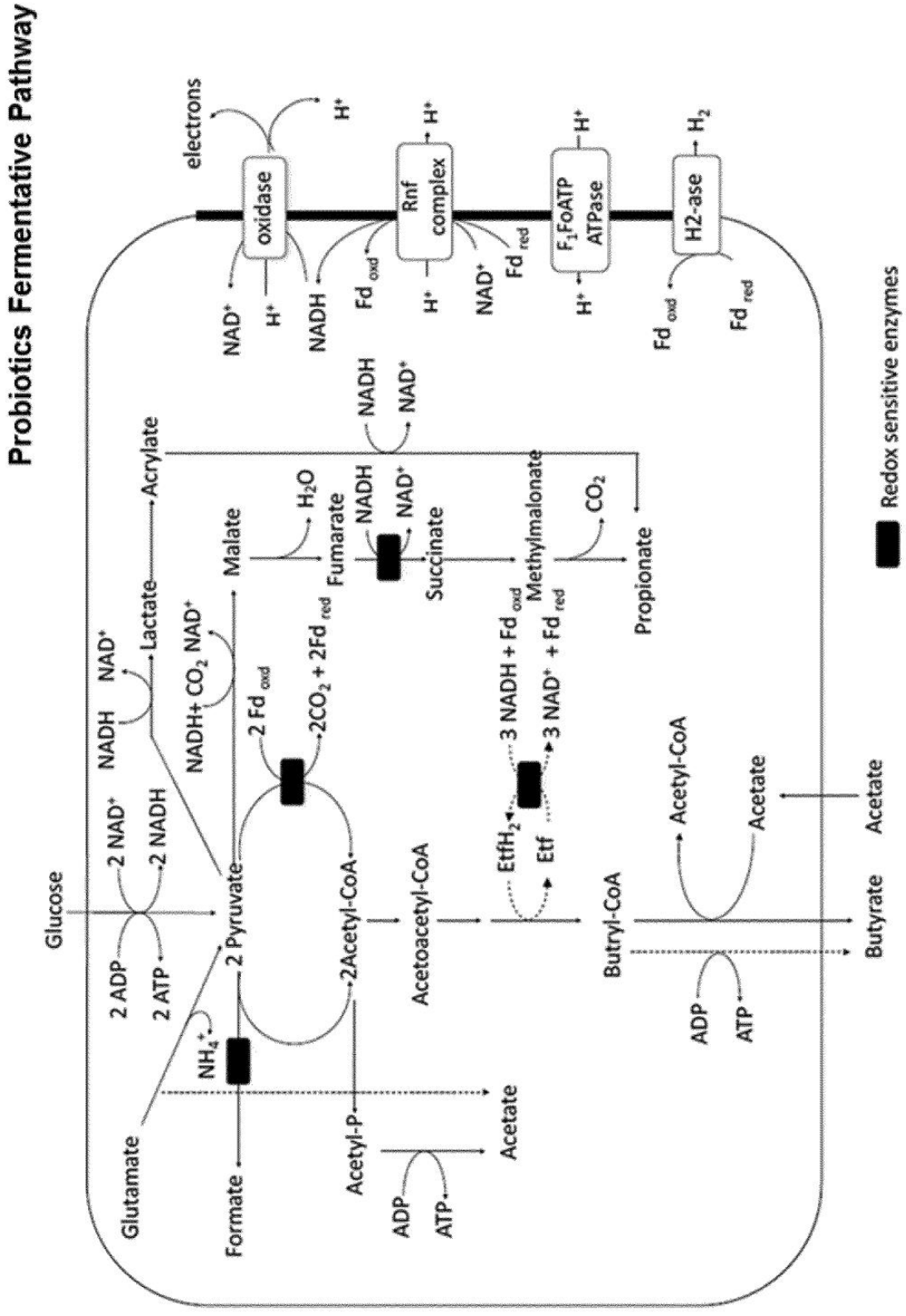

Probiotics Fermentative Pathway
electrons
oxidase
Rnf complex
F1FoATP ATPase
H2-ase
H+
H2
NAD+
NADH
Fd oxd
Fd red
Glucose
2 NAD+
2 NADH
2 ADP
2 ATP
2 Pyruvate
Lactate
Acrylate
Malate
H2O
Fumarate
Succinate
Propionate
Methylmalonate
CO2
NADH+ CO2 NAD+
2 Fd oxd
2CO2 + 2Fd red
3 NADH + Fd oxd
3 NAD+ + Fd red
EtfH2
Etf
2Acetyl-CoA
Acetoacetyl-CoA
Butryl-CoA
Acetyl-CoA
Acetate
Butyrate
Glutamate
NH4+
Formate
Acetyl-P
ADP
ATP
Redox sensitive enzymes

FIGURE 3

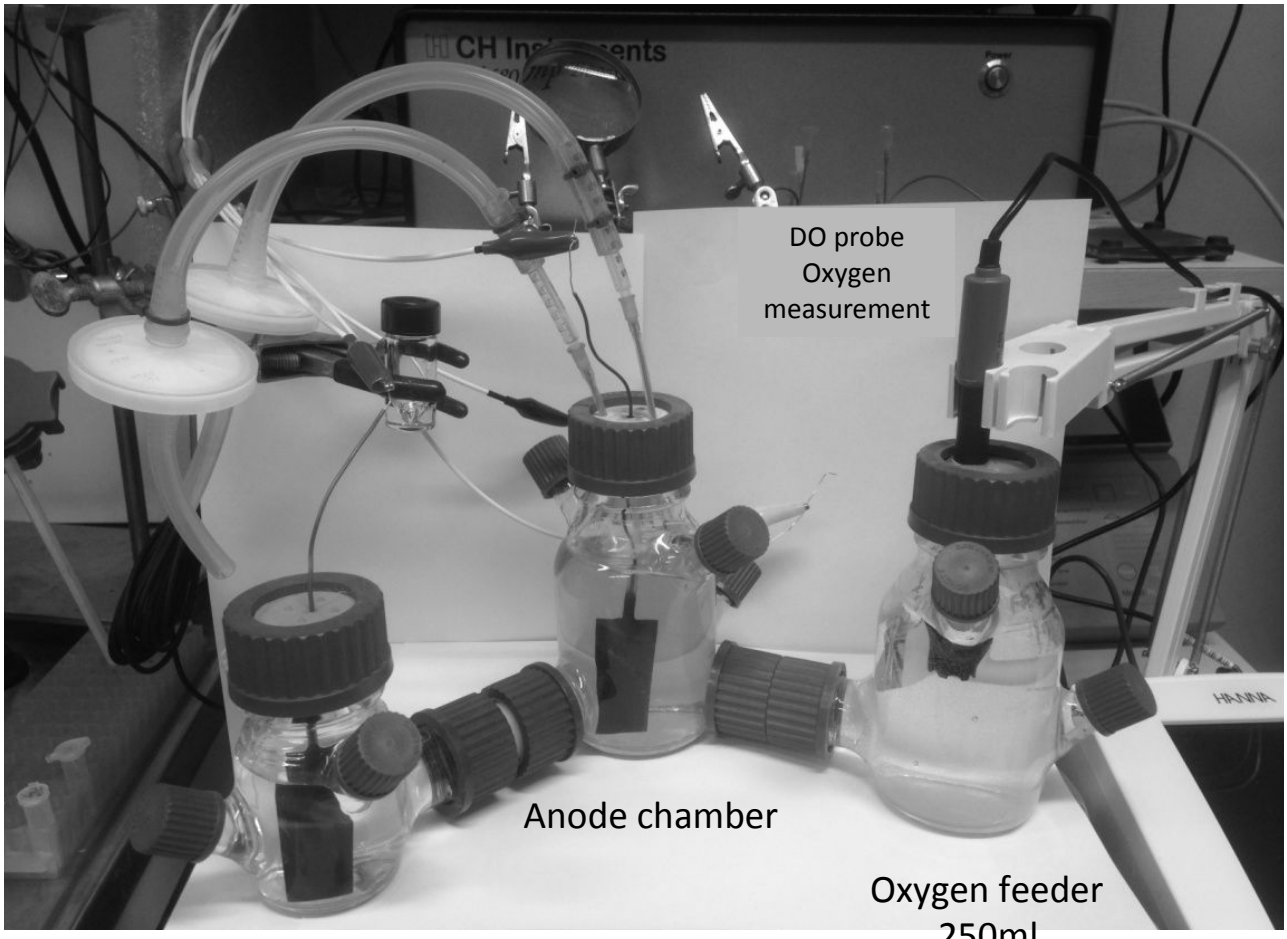

DO probe
Oxygen
measurement
Anode chamber
Oxygen feeder
250ml
Cathode chamber

Figure 4 a

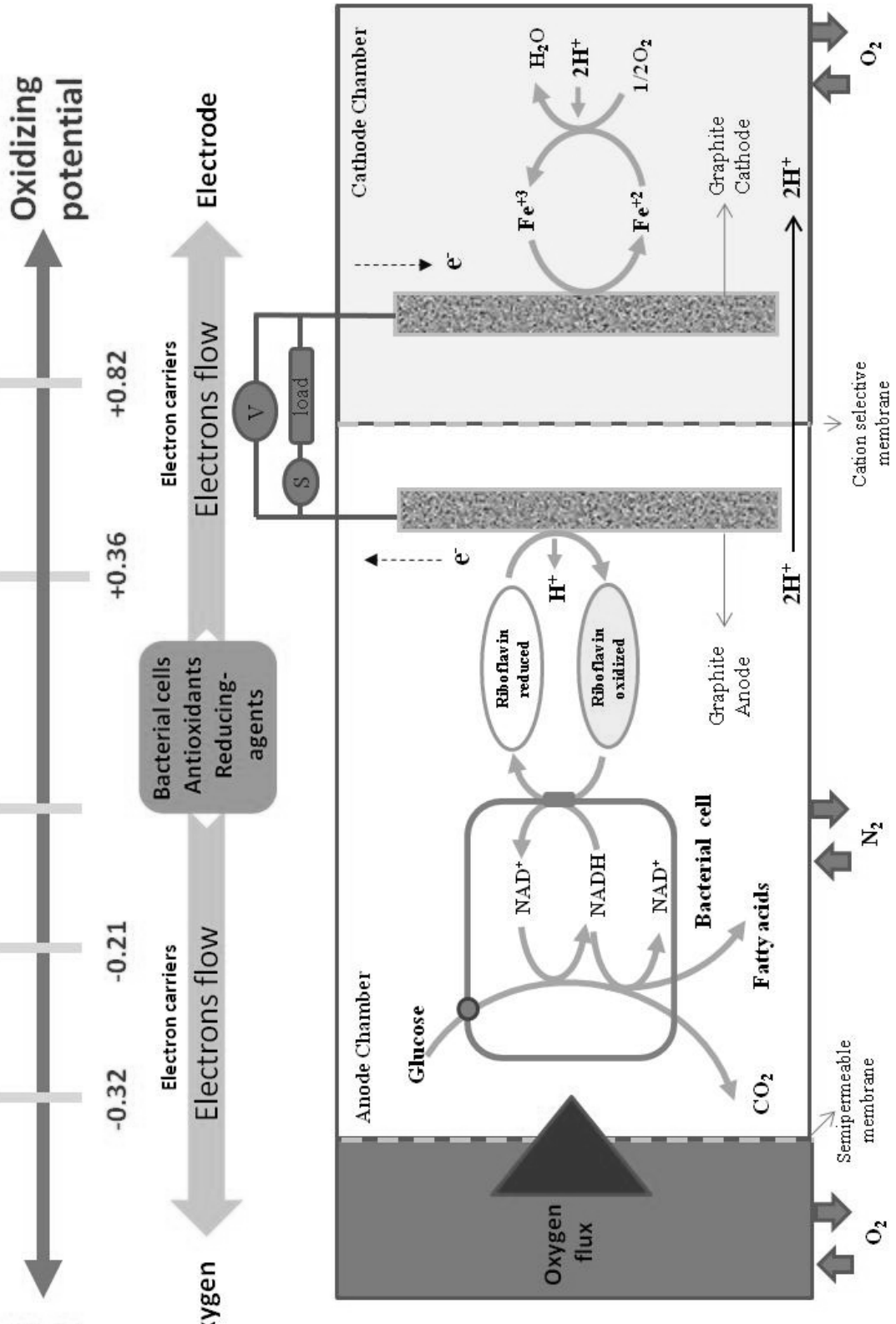

5/12
Oxidizing potential
Reducing potential
NADH
Riboflavin
Potassium ferricyanide
Oxygen
-0.32
-0.21
+0.36
+0.82
Electron carriers
Electrons flow
Electrode
Bacterial cells
Antioxidants
Reducing-agents
Oxygen
Cathode Chamber
Anode Chamber
Glucose
NAD+
NADH
NAD+
Bacterial cell
Fatty acids
CO2
Riboflavin reduced
Riboflavin oxidized
H+
e-
Fe+3
Fe+2
H2O
2H+
1/2O2
Graphite Cathode
Graphite Anode
Cation selective membrane
Semipermeable membrane
Oxygen flux
V
load
S
O2
N2

Figure 4 b

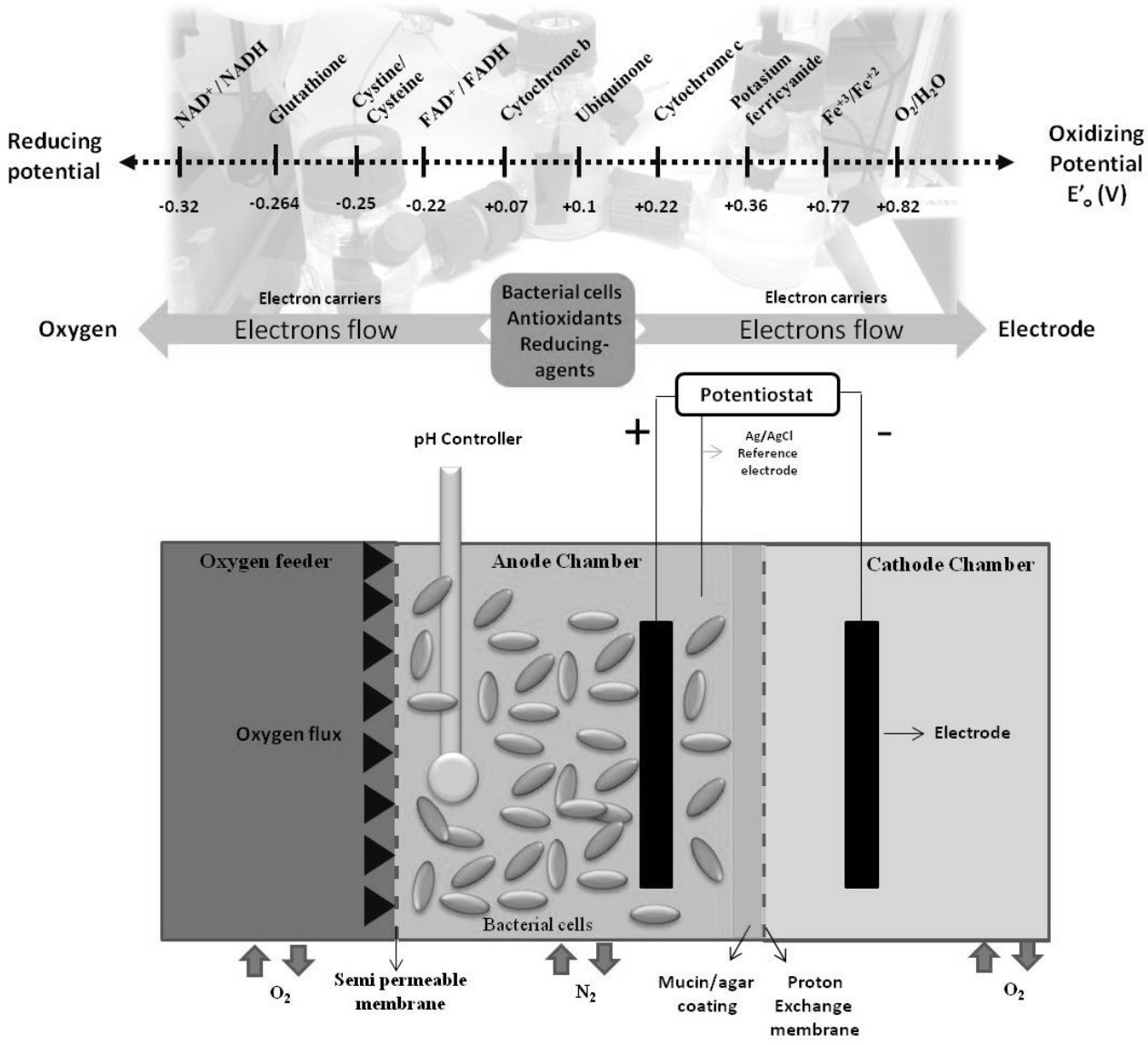

Reducing potential
NAD+ / NADH
Glutathione
Cystine/ Cysteine
FAD+ / FADH
Cytochrome b
Ubiquinone
Cytochrome c
Potasium ferricyanide
Fe+3/Fe+2
O2/H2O
-0.32
-0.264
-0.25
-0.22
+0.07
+0.1
+0.22
+0.36
+0.77
+0.82
Oxidizing Potential E'o (V)
Oxygen
Electron carriers
Electrons flow
Bacterial cells Antioxidants Reducing-agents
Electron carriers
Electrons flow
Electrode
Potentiostat
pH Controller
+
Ag/AgCl Reference electrode
-
Oxygen feeder
Anode Chamber
Cathode Chamber
Oxygen flux
Electrode
Bacterial cells
O2
Semi permeable membrane
N2
Mucin/agar coating
Proton Exchange membrane
O2

Figure 4 c

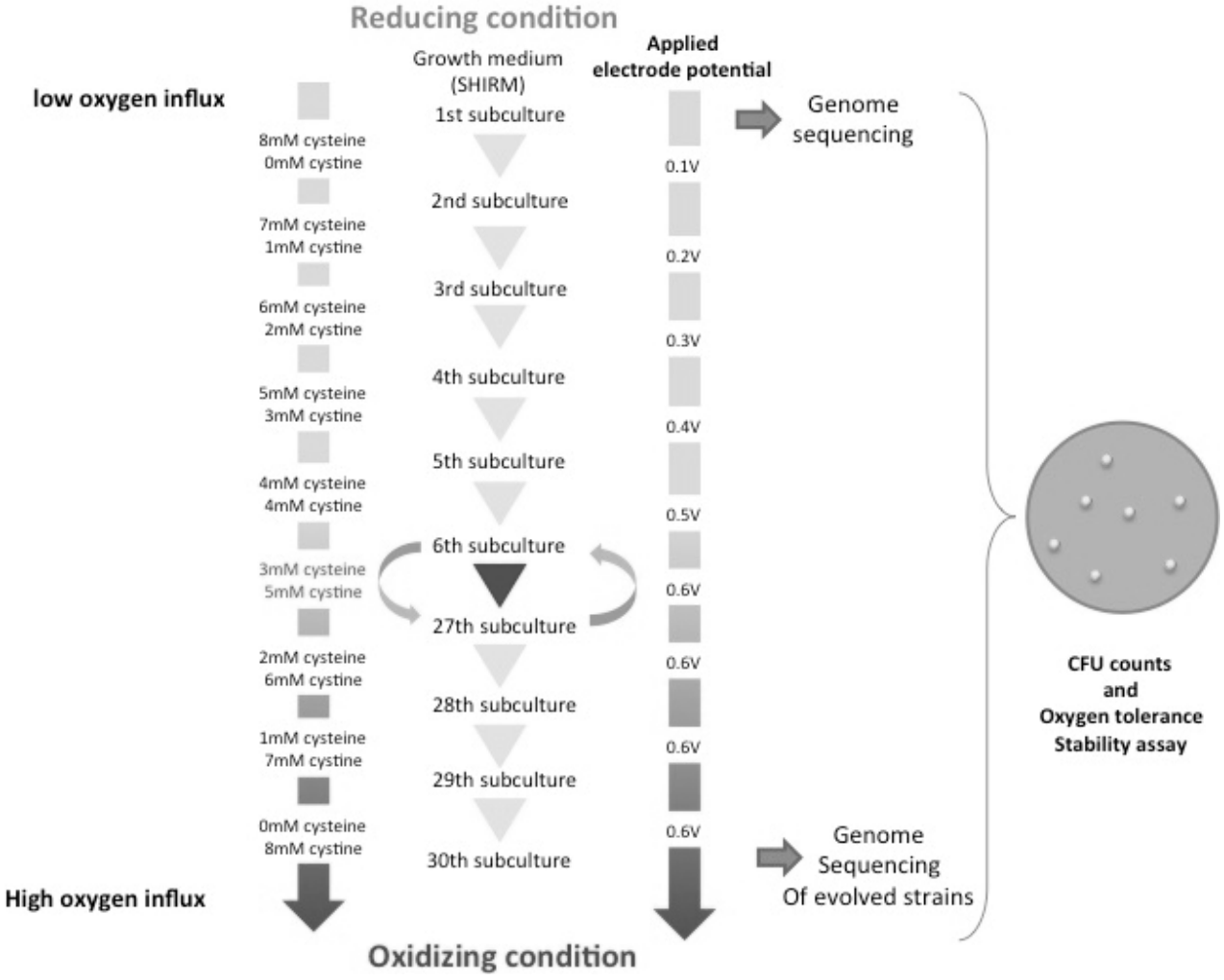

Reducing condition
Growth medium
(SHIRM)
Applied
electrode potential
low oxygen influx
8mM cysteine
0mM cystine
1st subculture
0.1V
Genome
sequencing
7mM cysteine
1mM cystine
2nd subculture
0.2V
6mM cysteine
2mM cystine
3rd subculture
0.3V
5mM cysteine
3mM cystine
4th subculture
0.4V
4mM cysteine
4mM cystine
5th subculture
0.5V
3mM cysteine
5mM cystine
6th subculture
0.6V
27th subculture
2mM cysteine
6mM cystine
0.6V
28th subculture
1mM cysteine
7mM cystine
0.6V
29th subculture
0mM cysteine
8mM cystine
0.6V
30th subculture
Genome
Sequencing
Of evolved strains
High oxygen influx
Oxidizing condition
CFU counts
and
Oxygen tolerance
Stability assay

Figure 5

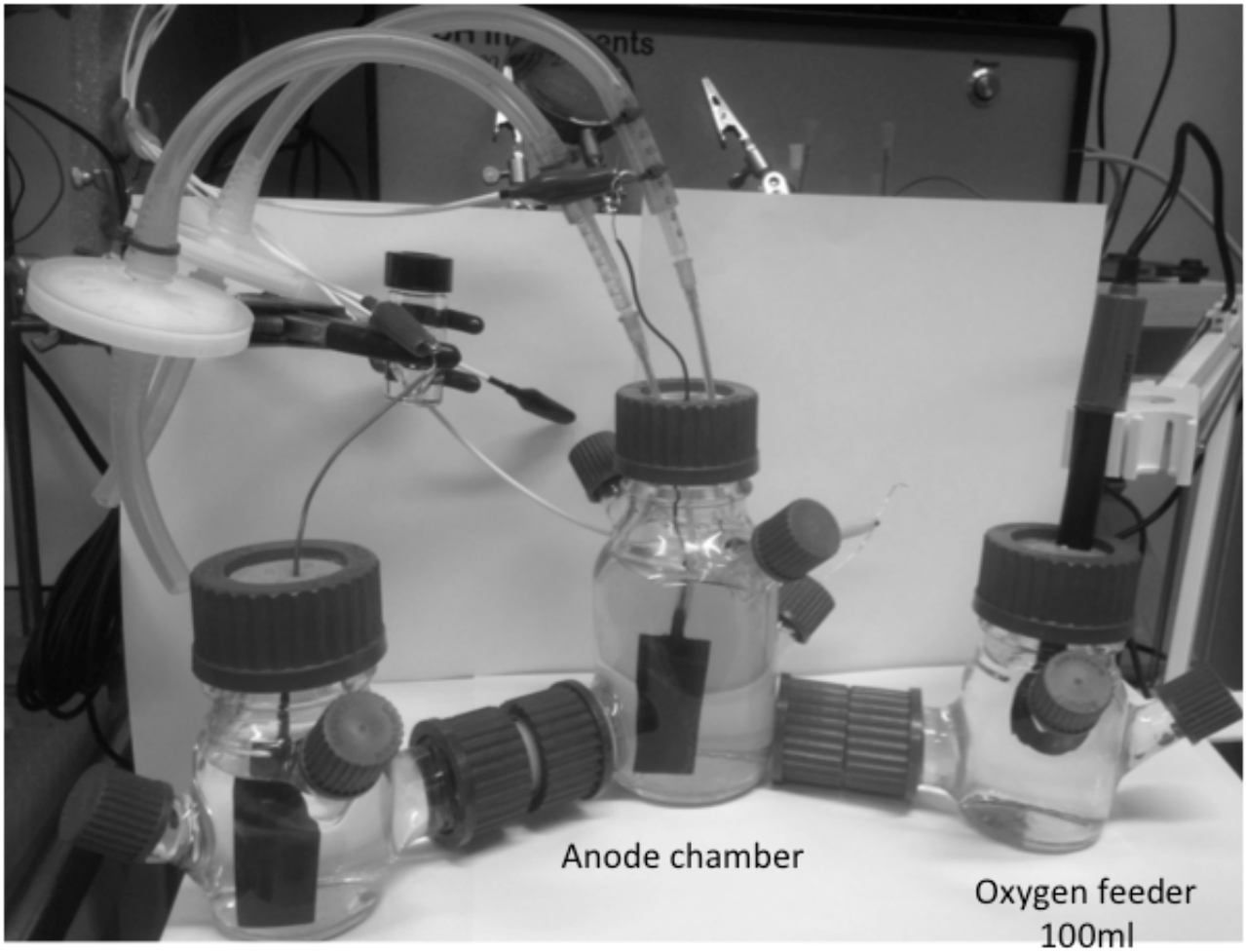


Figure 6

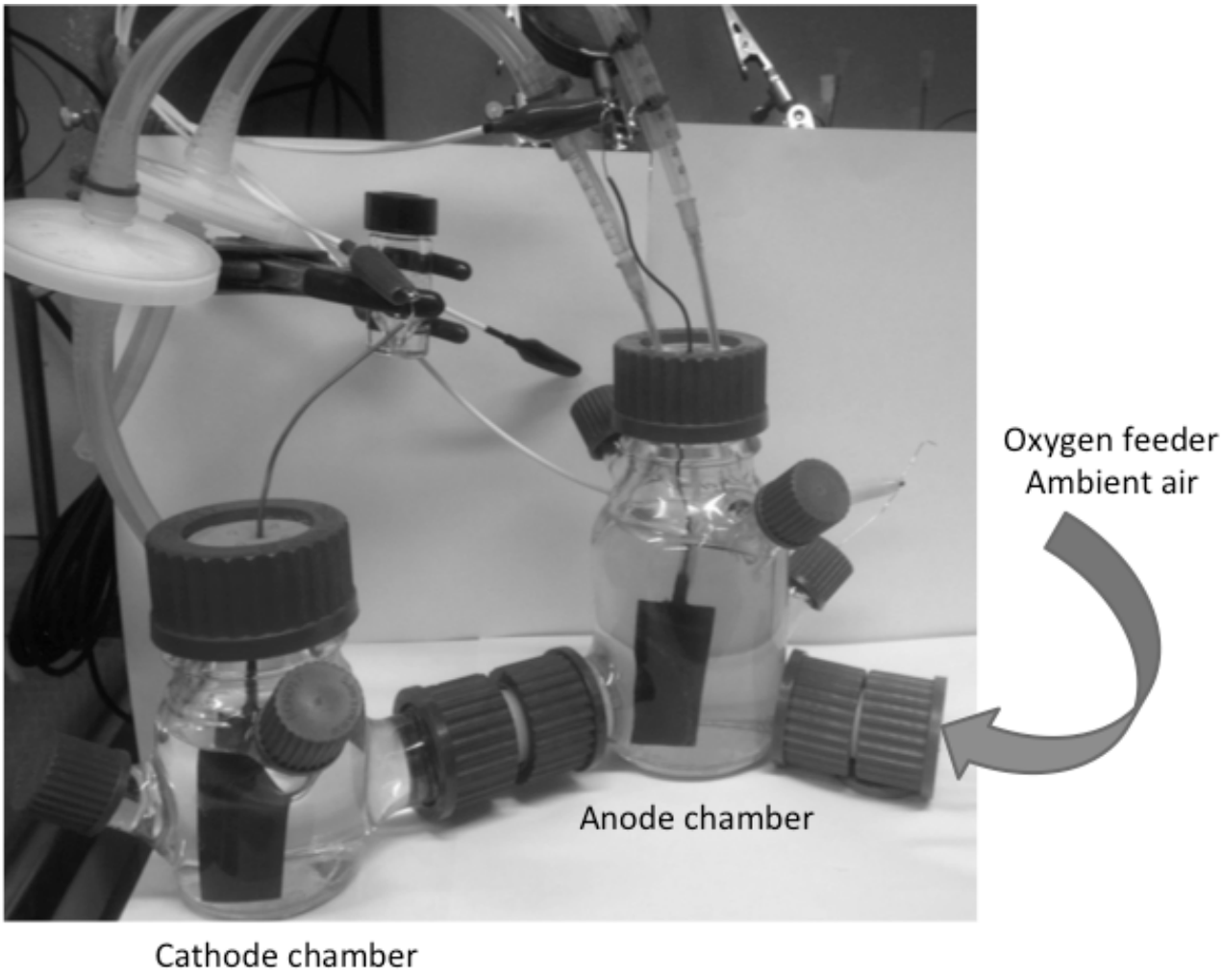


Figure 7

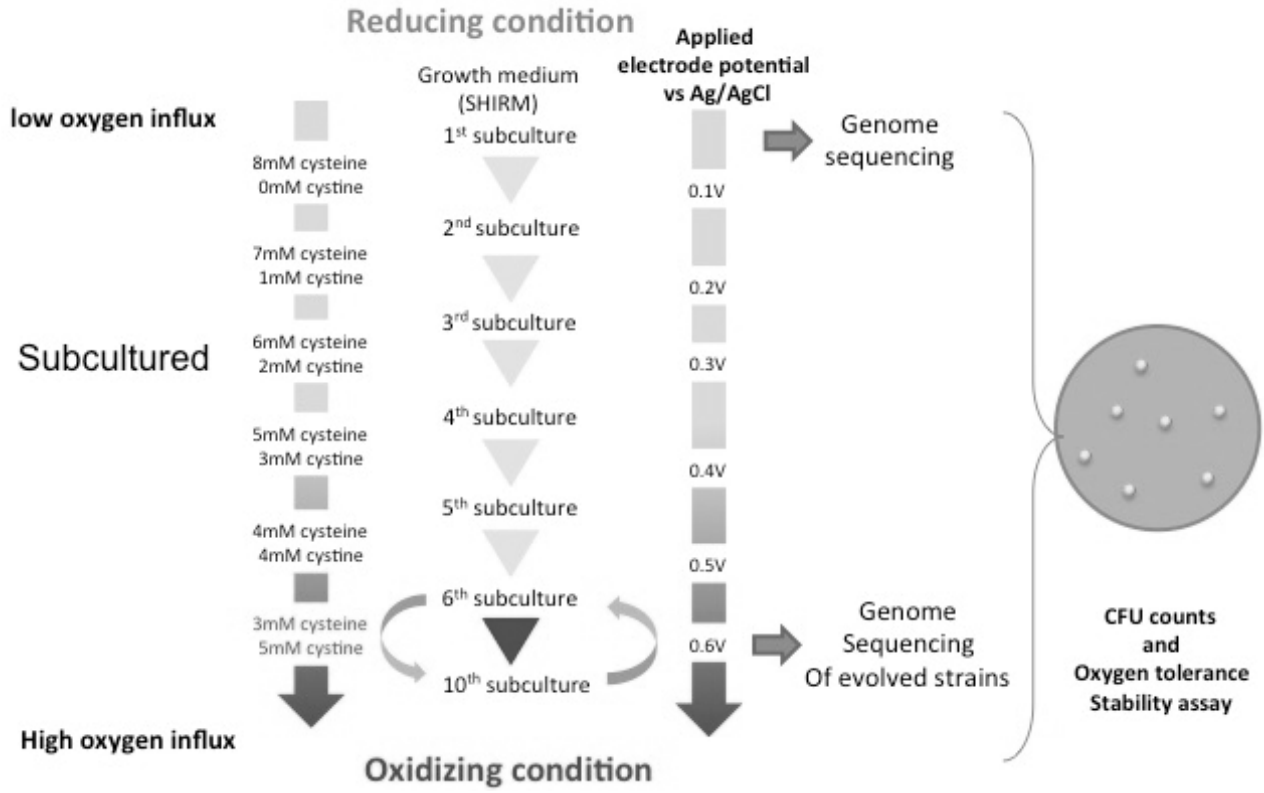

Reducing condition
Applied electrode potential vs Ag/AgCl
Growth medium (SHIRM)
low oxygen influx
1st subculture
8mM cysteine 0mM cystine
0.1V
Genome sequencing
2nd subculture
7mM cysteine 1mM cystine
0.2V
3rd subculture
Subcultured
6mM cysteine 2mM cystine
0.3V
4th subculture
5mM cysteine 3mM cystine
0.4V
5th subculture
4mM cysteine 4mM cystine
0.5V
6th subculture
3mM cysteine 5mM cystine
0.6V
Genome Sequencing Of evolved strains
10th subculture
CFU counts and Oxygen tolerance Stability assay
High oxygen influx
Oxidizing condition

Figure 8

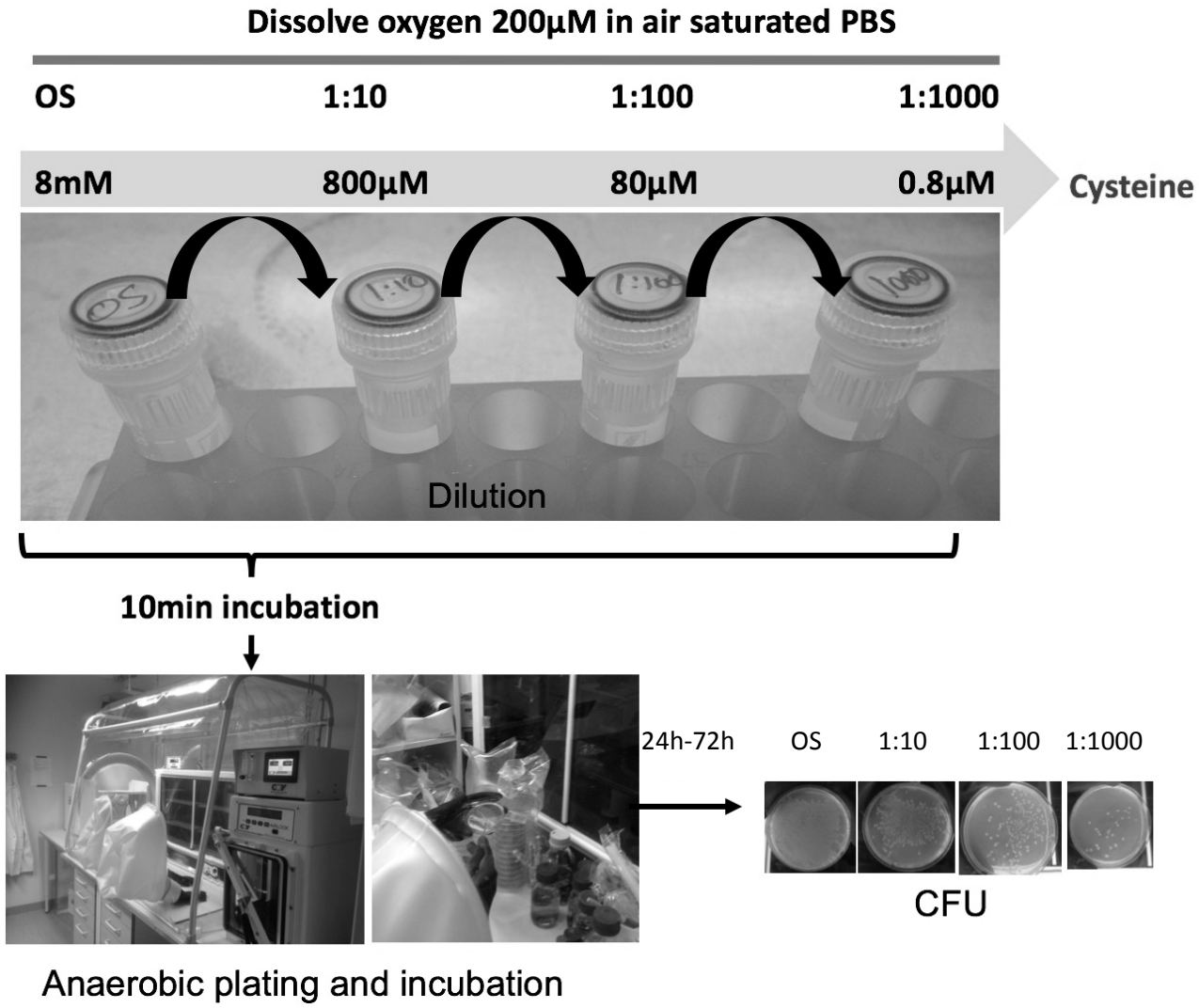

Dissolve oxygen 200µM in air saturated PBS
OS
1:10
1:100
1:1000
8mM
800µM
80µM
0.8µM
Cysteine
Dilution
10min incubation
24h-72h
OS
1:10
1:100
1:1000
CFU
Anaerobic plating and incubation

Figure 9

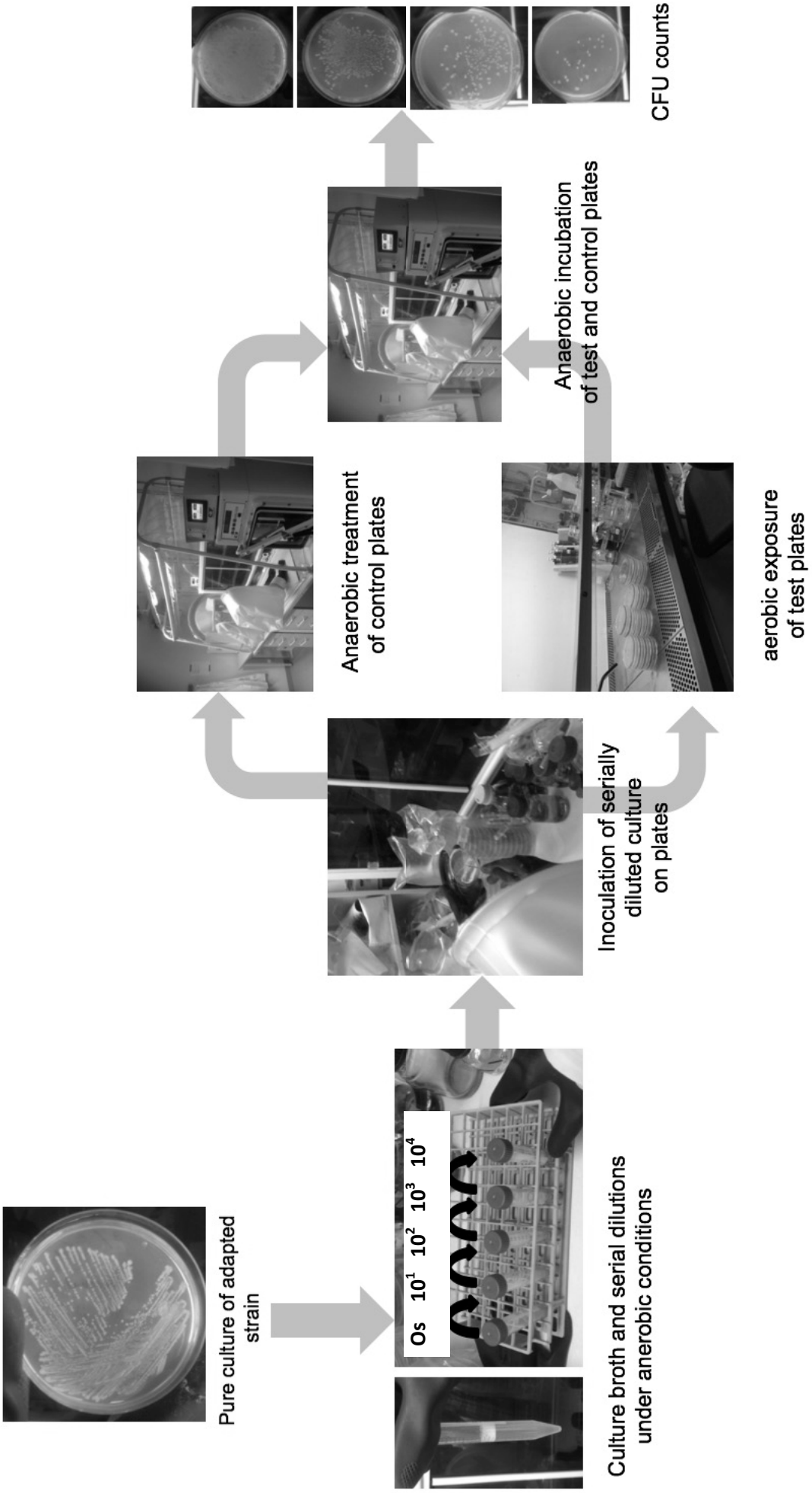

Pure culture of adapted strain
Os 10^1 10^2 10^3 10^4
Culture broth and serial dilutions under anerobic conditions
Inoculation of serially diluted culture on plates
Anaerobic treatment of control plates
aerobic exposure of test plates
Anaerobic incubation of test and control plates
CFU counts

Figure 10