A New qPCR Array for Cost-effective Quantification of MIC Microorganisms

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What is MIC?

Corrosion is the disintegration of metal through an unintentional chemical or electrochemical action, starting at its surface.

Microbially-influenced corrosion or MIC is a term applied to an increase in the rates corrosion reactions which can result from the growth and activity of microorganisms on metal surfaces

Corrosion of metals

- Corrosion results from the tendency of metals (some more than others) to be oxidized when in contact with water. Oxidation is a loss of electrons.

The reaction undergone by the metal is then:

$$M \rightarrow M^{+n} + ne^-$$

- The site of this reaction on the metal surface is called the **anode** of the corrosion electrochemical cell.

Corrosion of metals

- If one species is oxidized another must be reduced. There must be a simultaneous acceptance of the electrons generated at the anode at the **cathode** of the corrosion electrochemical cell.
- Common cathode reactions:
  - Reaction with hydrogen ions and forming hydrogen gas (acidic conditions):

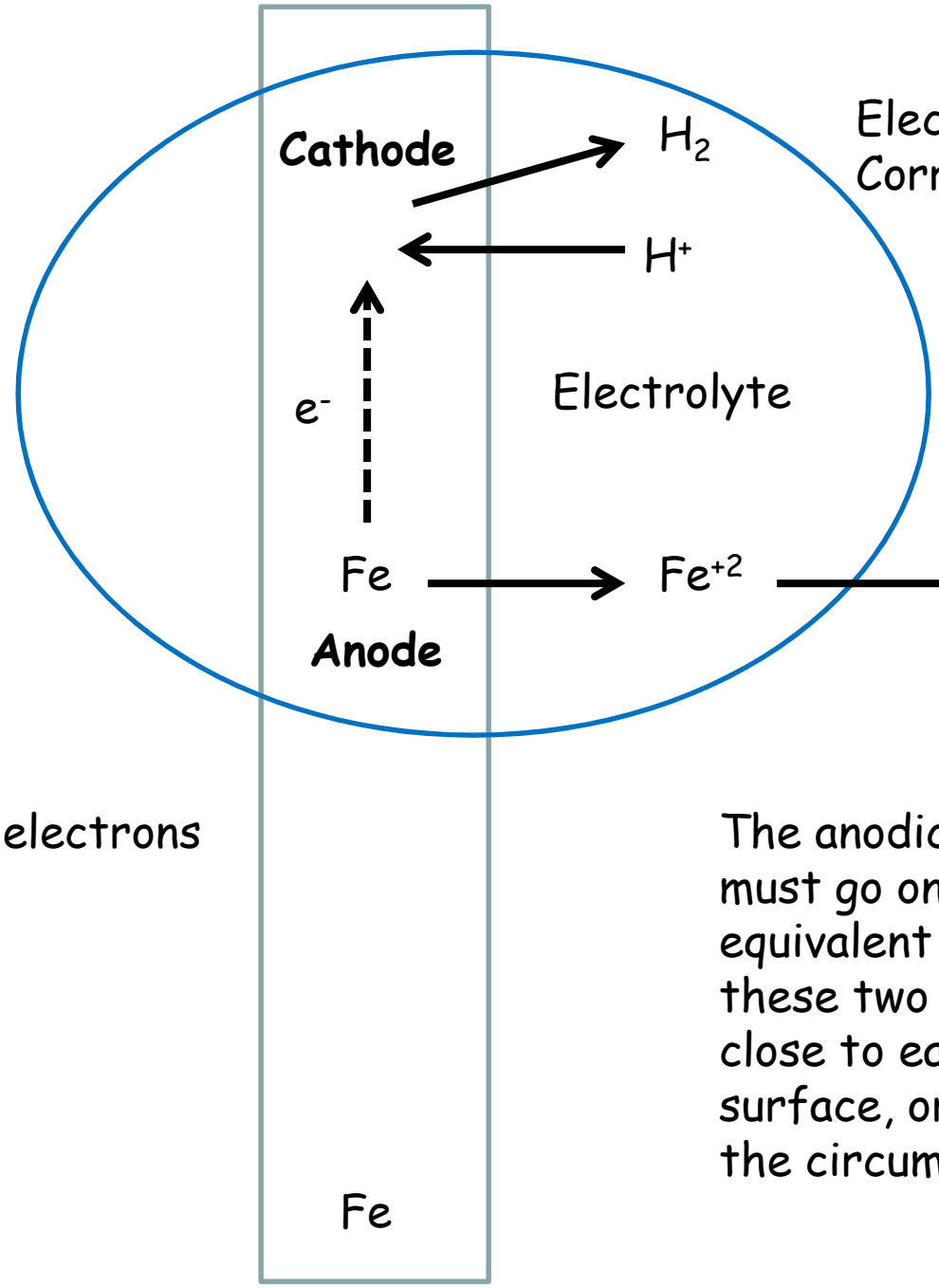
$$2H^+ + 2e^- \leftrightarrow H_2$$

  - Reduction of oxygen to form water (acidic conditions):

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

  - Reduction of oxygen to form water (neutral or alkaline condition):

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$$



Electrochemical Corrosion cell

Precipitation as corrosion products

e^- = electrons

The anodic and cathodic reactions must go on at the same time and at equivalent rates. The sites hosting these two processes can be located close to each other on the metal's surface, or far apart depending on the circumstances.

Another important cathode process

- Hydrogen gas will coat the cathode and isolate it from the water in a process called **polarization**. This breaks the connection between the cathode and the electrolyte and slows the corrosion process.

- Any process that consumes H_2 removes the coating and accelerates corrosion. This process is called **depolarization**.

- An example is the reaction of dissolved oxygen with the hydrogen gas surrounding the cathode:

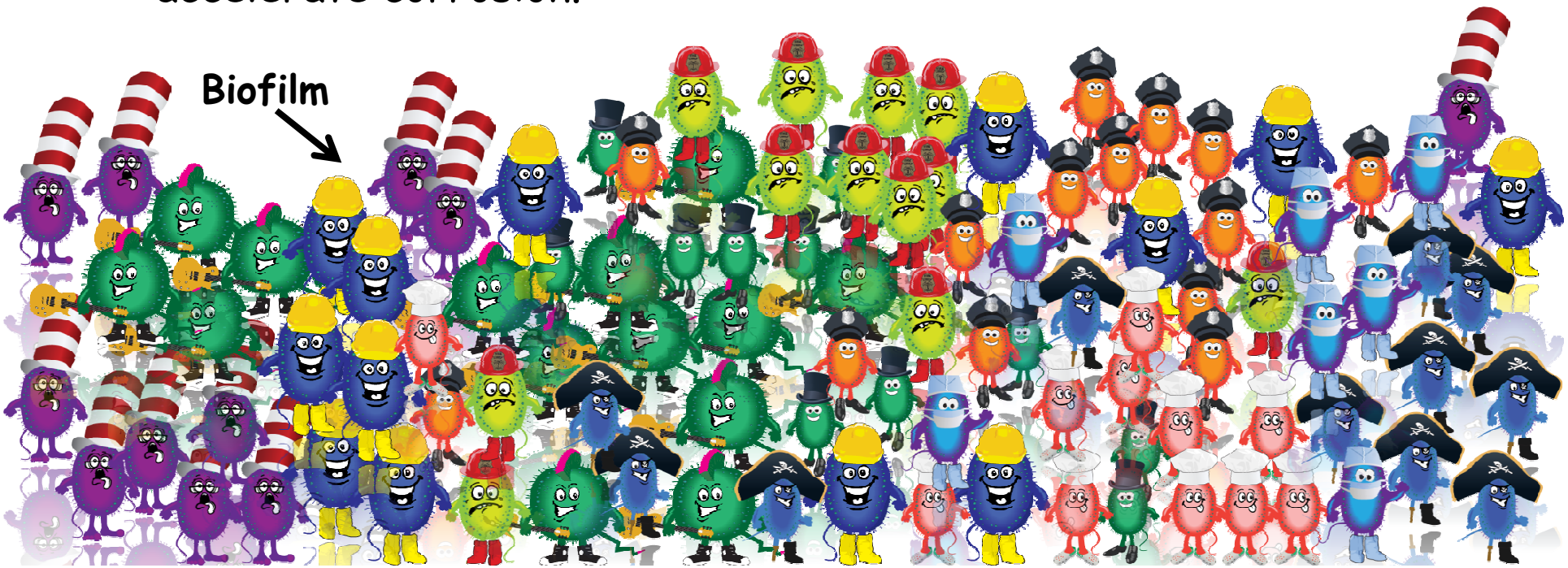
$$2H_2 + O_2 \leftrightarrow 2H_2O$$

How are microorganisms involved?

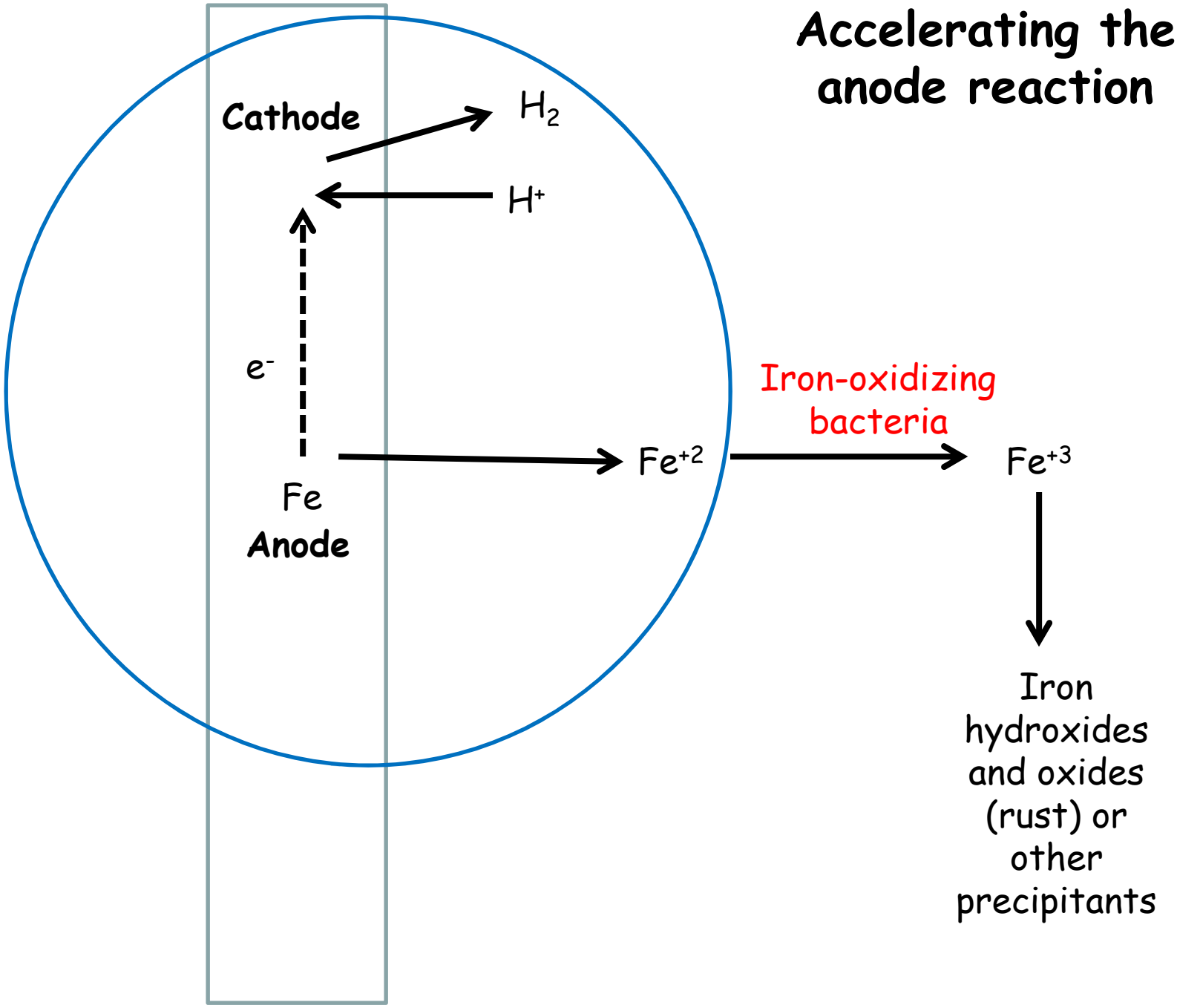
- Are there microbes in my system? Yes
- Microbes can tolerate a wide range of environmental conditions with respect to temperature, pH, and salinity
- Nutritional needs:
  - A source of carbon
    - Organic compounds (heterotrophs)
    - CO_2 (autotrophs)
  - A source of energy (oxidizable compounds)
    - Organic compounds
    - Reduced inorganic compounds: H_2, Fe^{+2}, sulfides, NH_4^+, etc.
  - Something to breathe (electron acceptor)
    - O_2 (aerobic)
    - NO_3^-(denitrifiers), Fe^{+3} (iron reducers), SO_4^{-2} (sulfate reducers), CO_2 (methanogens), MnO_2 (manganese reducers)

How are microorganisms involved?

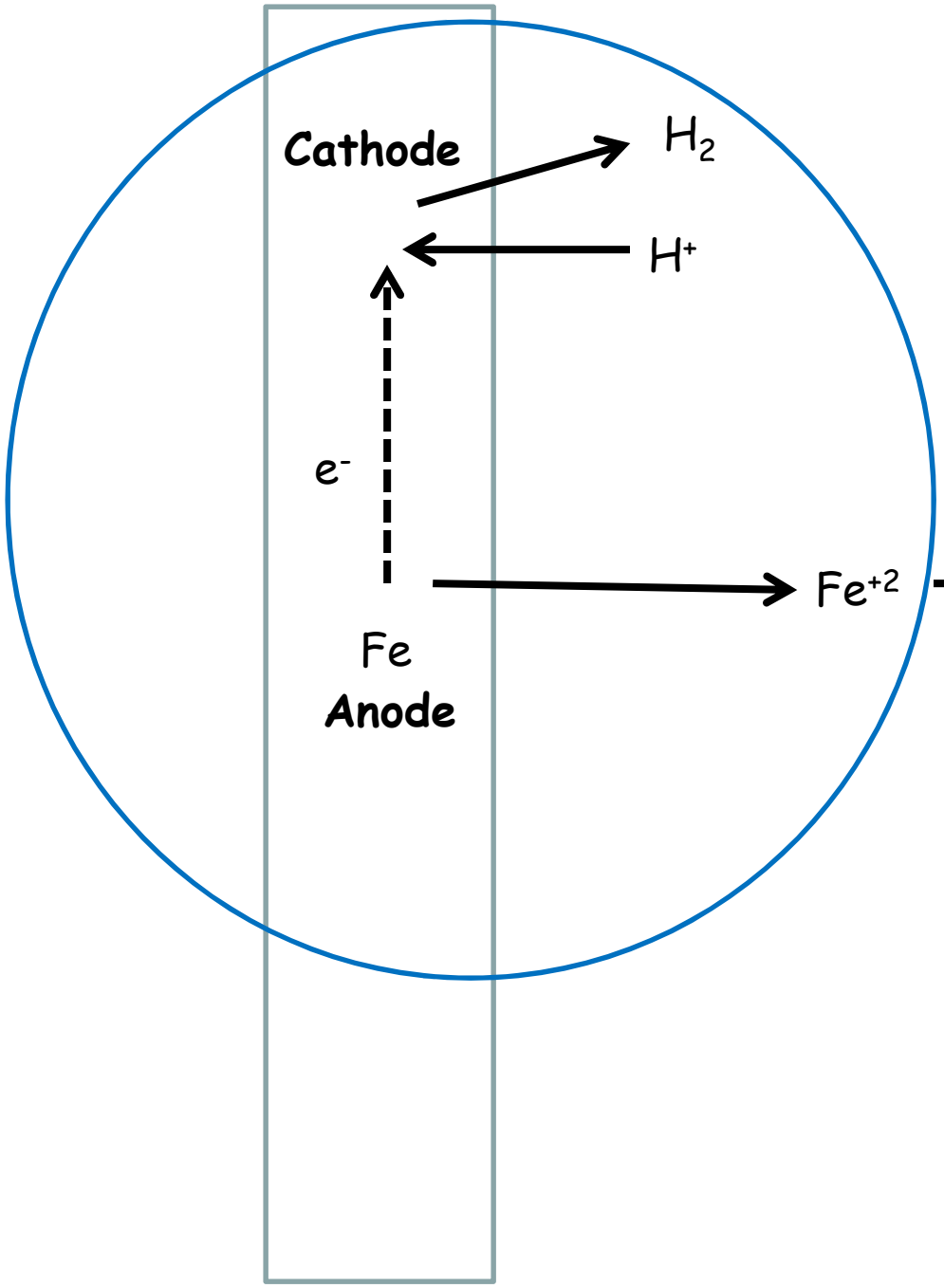
- Microorganisms prefer to live in biofilms adhering to a solid surface
- Complex communities encased in slime
  - Cross feeding
  - Protection
- Corrosion is a surface phenomenon. Biofilms can significantly influence the chemistry of the near surface environment and accelerate corrosion.



Accelerating the anode reaction



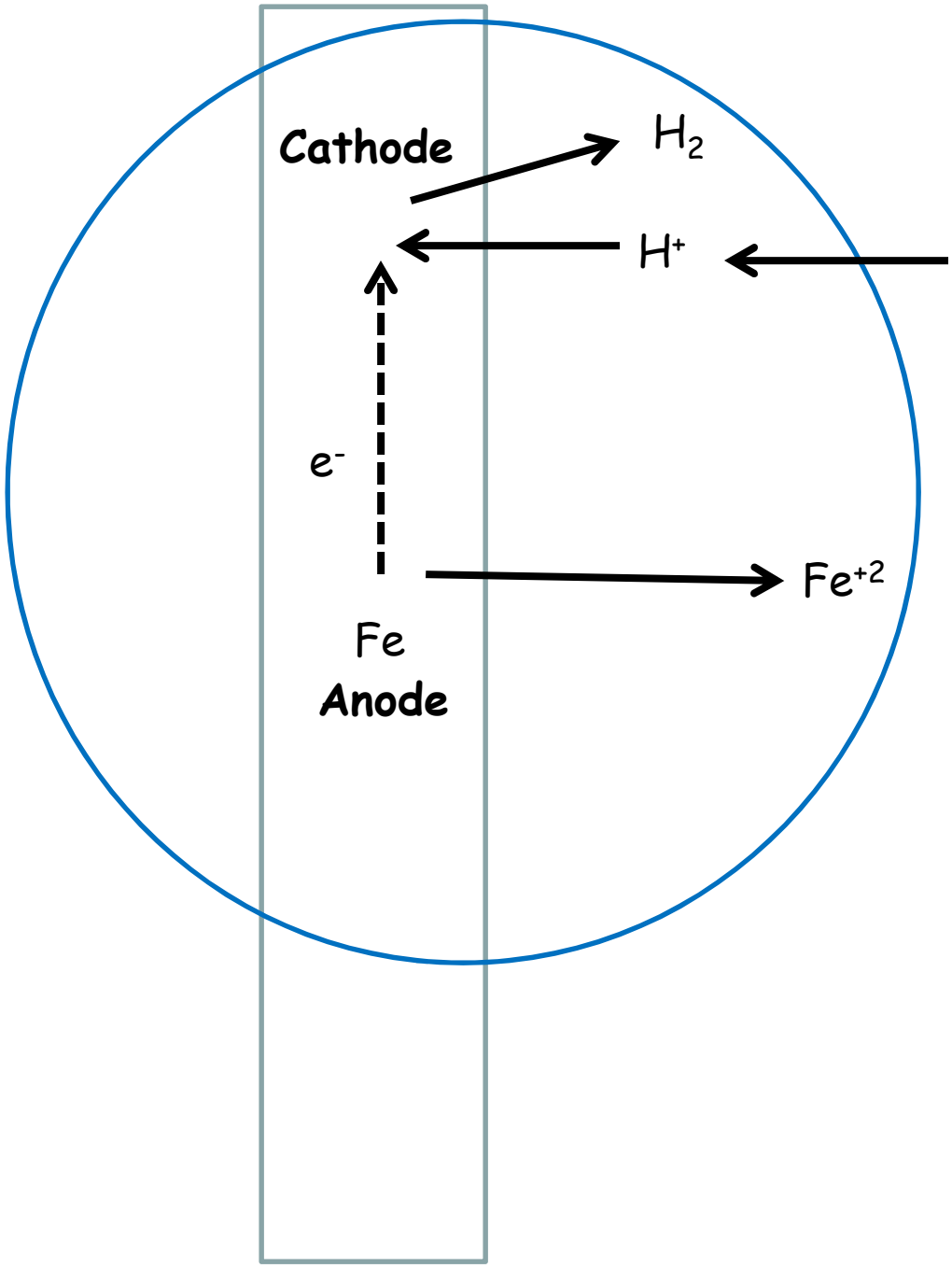
Accelerating the anode reaction



Sulfate-reducing bacteria (produce H_2S)

FeS

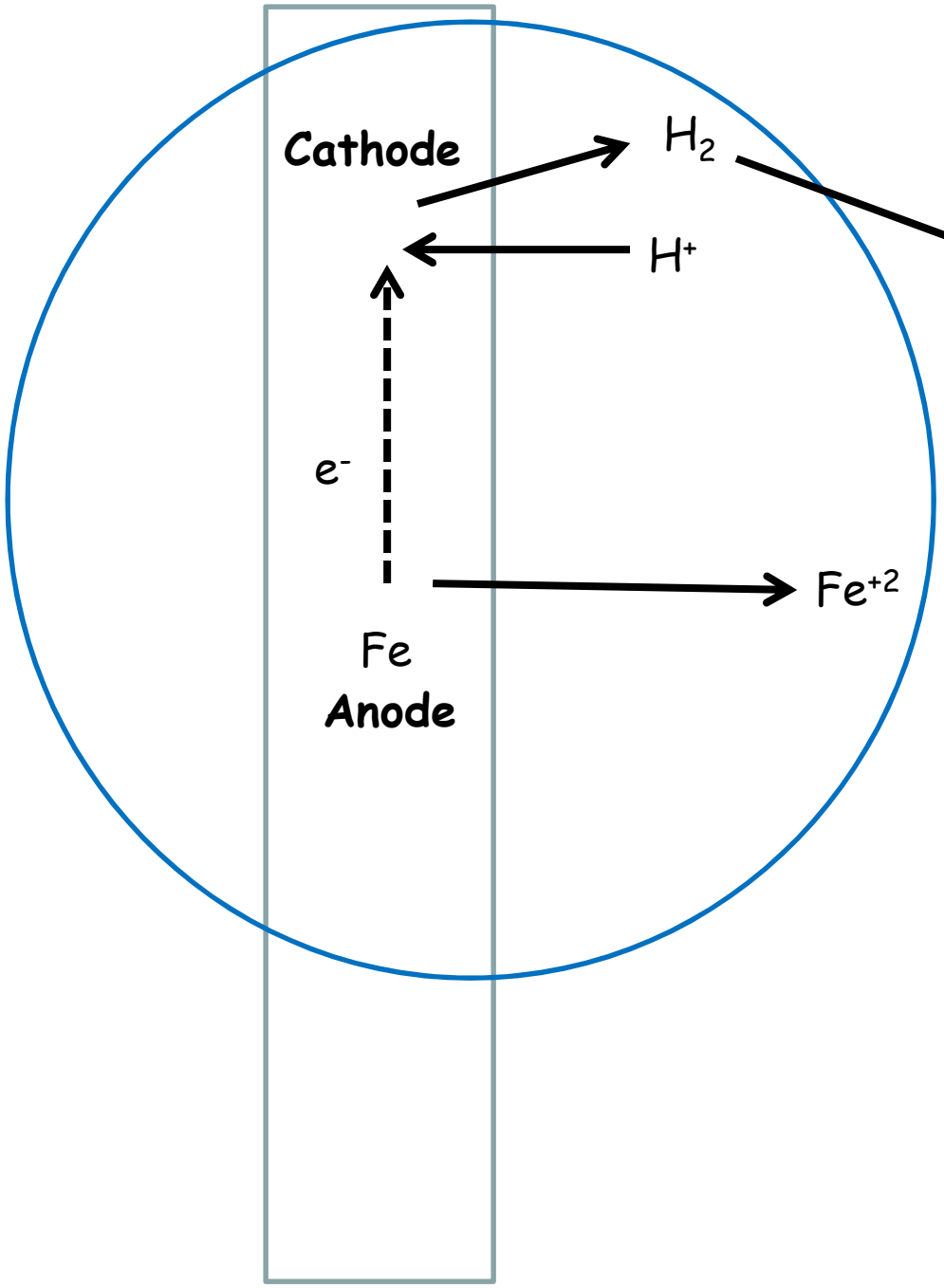
Black precipitant



Accelerating the cathode reaction

Acid producing bacteria (produce H_2S, organic acids, CO_2)

Accelerating the cathode reaction



H_2 consuming bacteria (also depolarizes the cathode)

Detecting and quantifying MIC

- Pitting
- Corrosion products
  - Fe^{+2}
  - Iron hydroxides and oxides
  - FeS (black precipitant)
- Presence and growth of MIC-correlating microorganisms
  - Sampling
    - Liquid samples
    - Corrosion coupons
    - Scrapings
  - Analysis
    - Growth based (bottle tests)
    - Molecular methods (qPCR)
- Products of microbial growth
  - Acids (volatile fatty acids, pH)
  - Alkalinity (CO_2)
  - Sulfides (S^{-2}, HS^-)
  - Methane
  - Slime
- In every case look for changes over time and in the direction of flow

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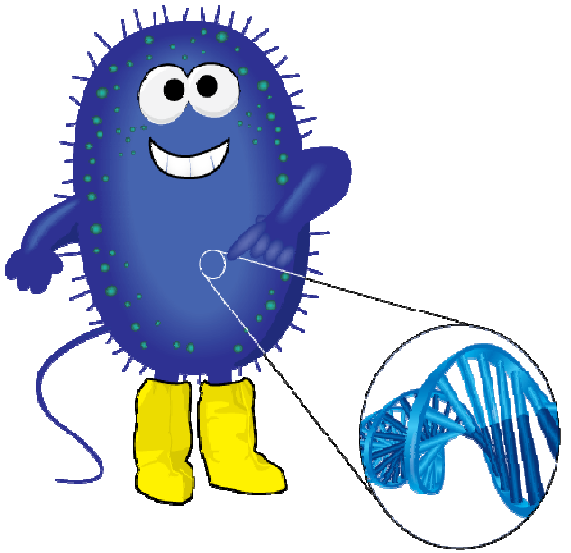
What is qPCR?

- A molecular biological tool to analyze microbial communities in environmental media
- It works by counting genes
- Genes are segments of DNA that code for the production of
  - an individual protein or enzyme (functional genes)
  - rRNA (taxonomic genes)
- See www.microbe.com for qPCR webinar



qPCR targets and MIC

- Total Eubacteria – general measure of microbial growth (**EBAC**)
- Iron oxidizers
  - Iron-oxidizing bacteria (**IOB**)
  - *Thiobacillus* spp. (**THIO**)
- Fe^{+2} precipitation
  - Sulfate-reducing bacteria (**APS**)
  - *Desulfovibrio* spp. (**DSV**)
  - *Archeoglobus* spp. (sulfate-reducing archaea) (**ARG**)

qPCR targets and MIC

- Acid-producing bacteria
  - *Clostridia* spp. (**CLO**)
  - *Bacteroides* spp. (**GENBAC**)
  - Sulfate-reducing bacteria (**SRB**)
  - *Archeoglobus* spp. (sulfate-reducing archaea) (**ARG**)
  - *Desulfovibrio* spp. (**DSV**)
  - Acetogens (**AGN**)
  - Sulfur-oxidizing bacteria (**SOB**)
  - *Thiobacillus* spp. (**THIO**)
  - *Geobacter* spp. (**GEO**)
  - *Cladosporium* (**CLAD**) – acid-producing fungi

qPCR targets and MIC

- Hydrogen consuming microorganisms
  - Methanogens (**MGN**)
  - *Shewenella putrifaciens* (**SHW**)
  - Sulfate-reducing bacteria (**SRB**)
  - *Desulfovibrio* spp. (**DSV**)
  - Acetogens (**AGN**)

qPCR targets and MIC

- Other microorganisms related to MIC
  - Denitrifiers (**NRB**) – use of nitrate to exclude SRB
  - Nitrogen fixers (**NFB**) – accelerate deterioration of nitrite-based corrosion inhibitors
  - NO producers – correlates with corrosion
    - Ammonia-oxidizing bacteria (**AOB**)
    - Nitrite-oxidizing bacteria **(NOB**)
  - Iron-reducing bacteria (**IRB**) – may protect against depolarization

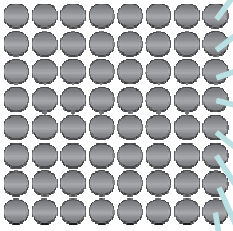
QuantArray approach: simultaneous analysis of numerous qPCR targets

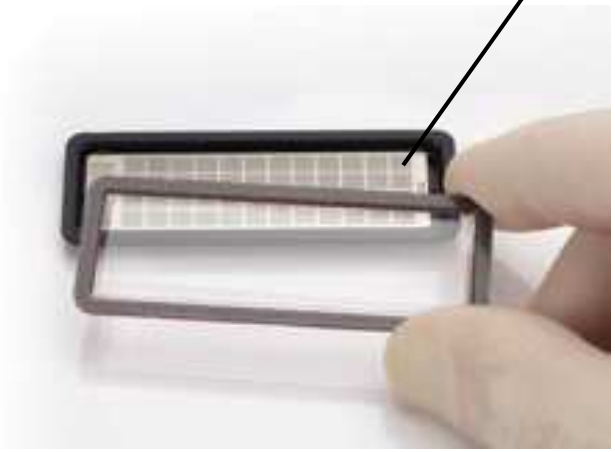
Sample Collection

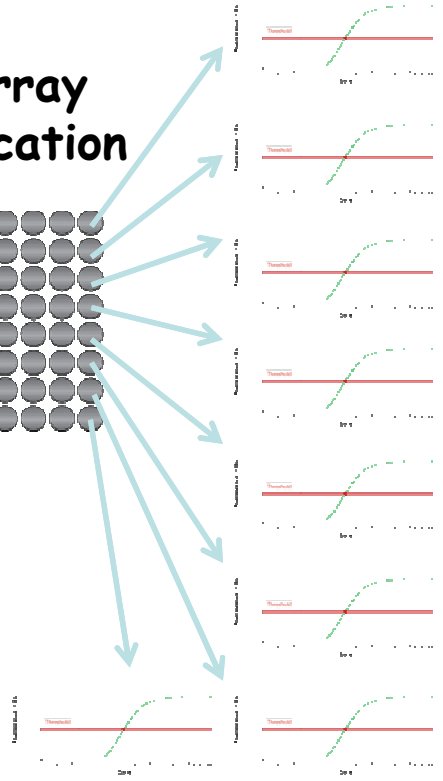
Water,
Pipe scrapings,
Corrosion coupon,
Pig return

DNA or RNA Extraction

SubArray Amplification





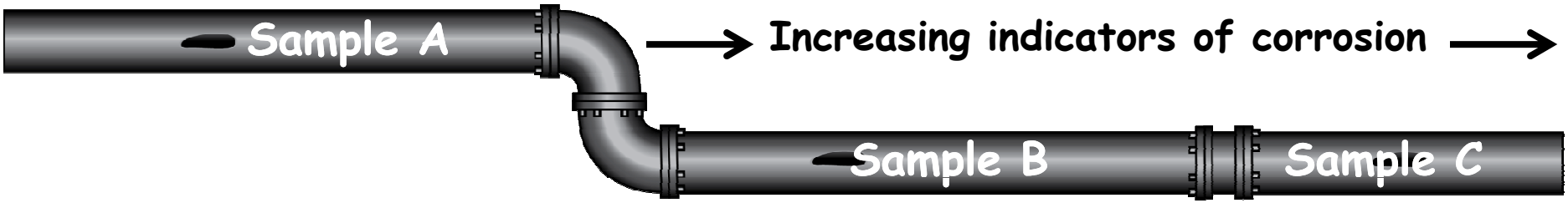


Quantification of multiple gene targets

Accurate quantification of all targets from a single analysis

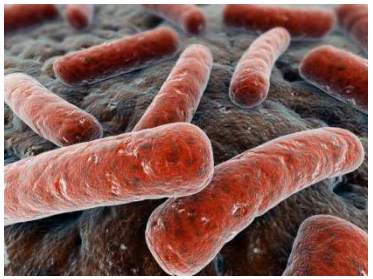
Example of using qPCR to investigate MIC

Samples were obtained at different points to investigate microbial growth along a pipeline.



Are microorganisms growing along the pipe?

If so, what populations are growing?



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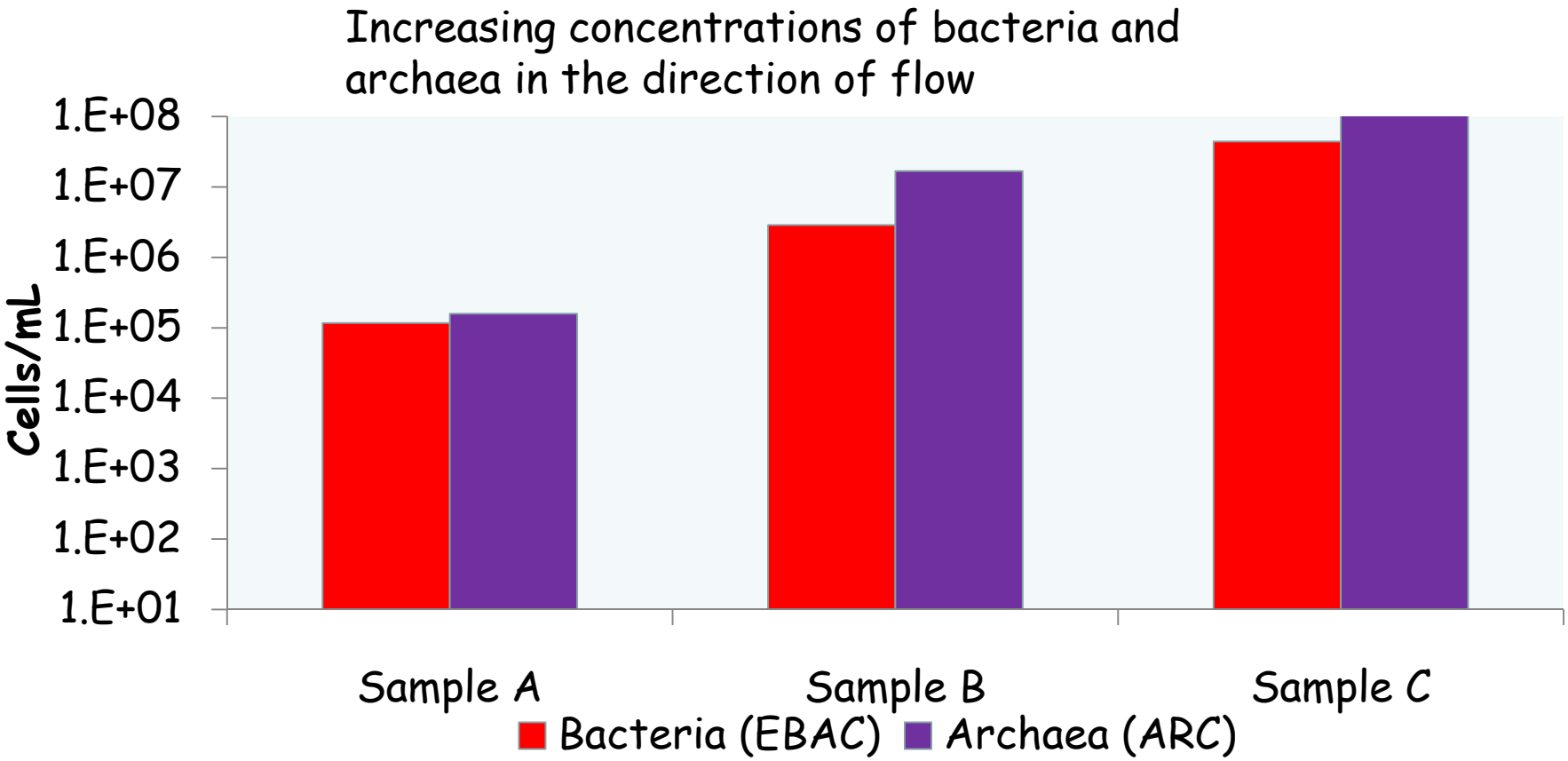

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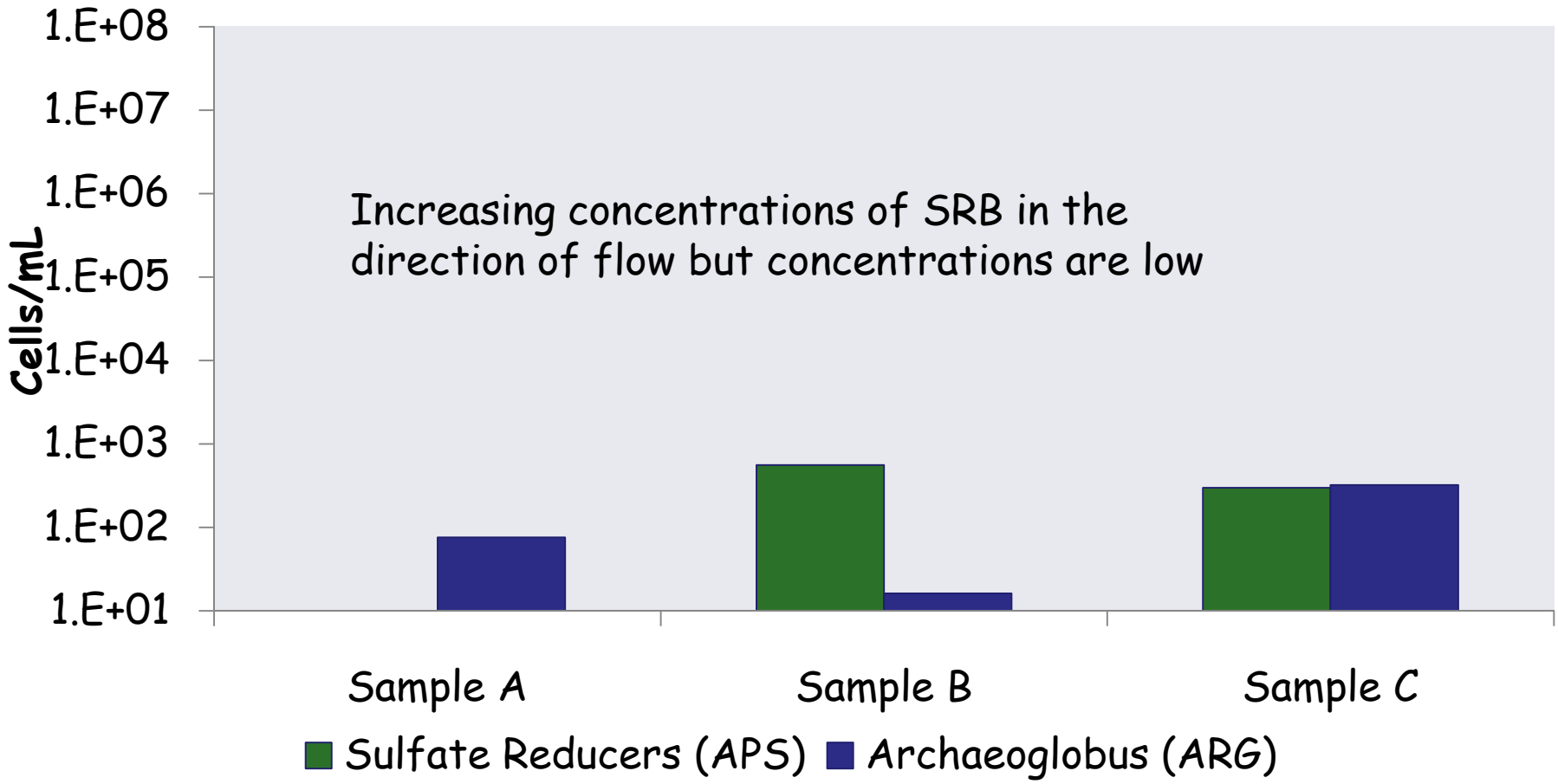

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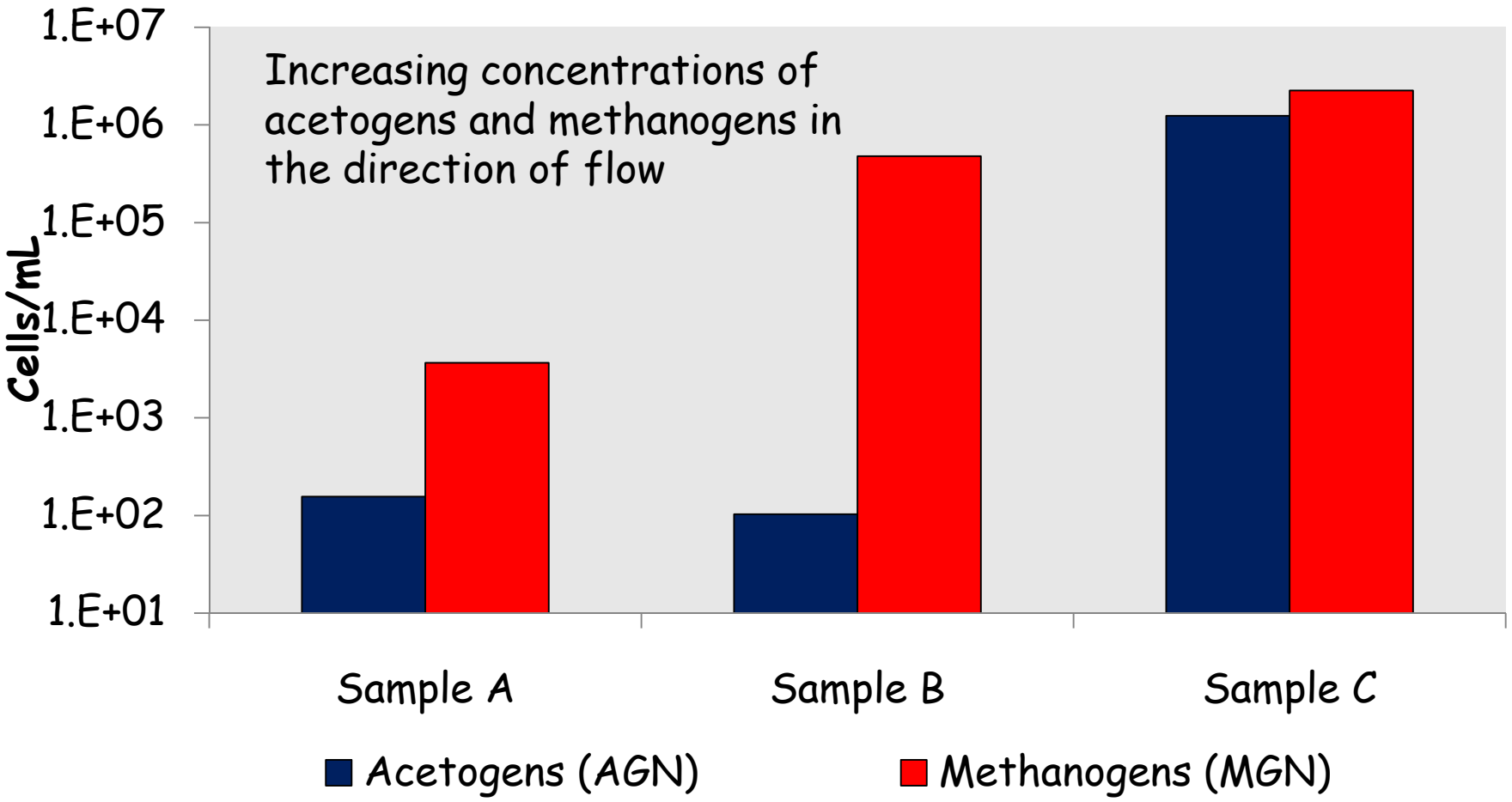

Potential MIC mechanisms

- Depolarization
  - Both methanogens and acetogens consume hydrogen
  - SRB may contribute
- Acid production
  - Acetogens produce acetic acid
  - Both groups are anaerobic suggesting possible growth of fermenters which can produce organic acids and CO_2
  - Some sulfate reduction but may be minor contributor