**Background**

Obligately anaerobic, Slow growing, Fastidious (1)

Dissimilatory reduction 🡪 hydrogen sulfide

Require free sulfate for growth. (2)

SRB require electron accepter for growth, usually sulfate. Sources for anion may be dietary or endogenous. high sulfated food = fermented beverages, dried fruits, white bread. (3)

SO4 2- reduction to produce H2S requires 8 e.

Step 1: SO4 2- activated using ATP and ATP sulfurylase so that it binds 🡪adenoside phophosulfate (APS) and elemental PPi

Step 2: Dissimilative: SO4 2- in APS reduced to sulfite (SO3 2-) using APS reductase. (2e in, AMP out)

Step 3: SO3 2- reduced to H2S via sulfite reductase. (6e in)

Provides H2S and free energy.

Electron transport chain generates proton motive force.

H+ moves across membrane/down gradient through ATPase 🡪 ATP synthesis

Capable of energy conservation by reducing elemental S and (source of electrons) 🡪 H2S.

Sulfur reducers cannot activate sulfate to APS.

Use elemental S instead. (Book pg 421-423)

Sulfur reducers also reduce e acceptors such as nitrate, ferrous iron, thiosulfate as alternative to elemental S. (Book pg 658)

Any sulfur compound with ox state above sulfide (-2) can be e acceptor

Dissimilatory: sulfate ion used as oxidant for degradation of organic material

H2S significant able to inhibit the growth of aerobic org.

Requires inorganic e acceptor.

Type of Carbon source used for reduction of acceptor varies by genus.

Acetate and Hydrogen are essential substrates.

Obligately anaerobic, Slow growing, Fastidious (1)

Obligate anaerobes lack catalase. (Lab manual pg 41)

Metabolic end product is sulfide, converted to H2S with external H+

Sulfide is corrosive/binds rapidly to metals. (4)

**Materials/Methods**

**Sources**

Sewage, industrial effluents, water, soil (1)

Found in periodontal pockets, gastrointestinal tract

Found in sea water, water sediments mud

Belongs in normal microflora of human oral cavity (5)

sediments, sewage sludge, and colons. (3)

**Isolation/Incubation/Media**

Chocolate agar

5 days, 37 degrees C

Not good in broth medium, better to use solid.

May take 5-7 days for a colony to appear. (1)

medium made by sterilizing separate solutions that were aseptically combined under anaerobic conditions.

All incubation in anaerobic chamber.

More details in article. (2)

Autoclaved at 121 C for 15 minutes.

Anaerobic = oxygen free, nitrogen headspace

Solution details in article. (3)

DRCA agar

TSN agar

Reduced-growth medium for SRB

Iron indicator for sulfide production

Samples incubated in anoxic chamber, 37 degrees C

3-7 days until black colonies

(5)

Anaerobic Methods.

Candle Jar. Burning convert oxygen to CO2 and Water.

Gas-Pak system. Chemical packet consumes oxygen when water is added.

Thioglcolate broth (Lab Manual pg 41)

**Identification**

Tiny, pinpoint, round colonies

Gram negative

Curved/rod-shaped

Single polar flagellum (1)

Identify using respiratory type, morphology, cultural aspects, stain affinity, gram stain

Further test: evidence of motility, production of only H2S, catalase test, sensitivity to kanamycin, colistin, vancomycin. (5)

Gas production visualized in Durham tubes.

Look for insoluble precipitate when combined with metal salts. (Lab Manual pg 57)

Catalase Activity.

3% H2O2 on slide. Bubbles🡪catalase. Else 🡪obligate anaerobe (Lab Manual pg 61)

Media for sulfate reducers should include FeSO4, to see FeS precipitate.

SIM (Sulfide Indole Motility) Medium. Dark tube🡪presence of H2S. (Lab Manual pg 63)

TSI (Triple Sugar Iron Agar) to test for H2S production. Lighter colors 🡪presence of H2S (Lab Manual pg 67)

Anaerobic chemostat enrichments. Chemostat = continuously add medium.

Sodium acetate as carbon source (2g/l)

Inoculated with 50g freshly collected sediment.

2 day intervals, 10ml samples aseptically removed.

Aliquot volumes transferred to agar shake dilution tubes (same medium)

Substrate Utilization Tests.

25 degrees C, up to 7 days

Gram stain at the end to confirm absence of contaminants.

Chomostat enrichments using acetate as carbon source were successful, target species becoming dominant after 12-14 days

Table 1: morphology of acetate using-sulfate reducing bacteria.

(6)

Found in sediment.

Use enrichment/mixed inocula or chemostat methods.

Batch culture: closed. No need to add. Simpler.

However, might need chemostat.

SRB are slow growing. Unable to compete in enrichment system?

Anaerobic plating do not distinguish bw different genera

Found in marine/estuarine sediments.

Saline ponds.

Especially where habitat contaminated by alternative nutrients from sewage effluent.

Anaerobic, but found in low conc in oxic conditions.

polluted environments such as spoiled food, sewage plants (4)

Presence indicated by strong blackening due to precipitation of FeS.

Morphological criteria and substrate utilization tests to identify (2)

2 categories for those that use lactate for carbon/energy:

spore-forming straight

curved rods

Activity can be detected to odour of H2S

Identify using motility tests, Gram reaction, cell size, morphology

(4)

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