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An MPN Method for the Enumeration of Iron-Reducing Bacteria

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Iron-Reducing Bacteria

- Use ferric iron as a terminal electron acceptor
- Large number of bacterial species can reduce iron
- Iron-reducers are widely distributed
- Simple organic compounds are used as electron donors by iron-reducers (e.g. ethanol, propanol, pyruvate, lactate, butanol, acetate formate, and BTEX compounds)
- Hydrogen is also used as an electron donor

Electron Acceptors

- O_2 Þ H_2O
- NO_3^- Þ N_2
- Mn^{4+} Þ Mn^{2+}
- NO_3^- Þ NH_4^+
- Fe^{3+} Þ Fe^{2+}
- SO_4^{2-} Þ HS^-
- CO_2 Þ CH_4
- H^+ Þ H_2

Reduction Rates of Various Forms of Iron

Ferric citrate

amorphous Fe(III) oxyhydroxides

more crystalline Fe oxides

Iron-Reducing Bacteria

- *Pseudomonas* spp.
- *Clostridium* spp.
- *Geobacter* spp.
- *Shewanella* spp.
- *Acidophilium cryptum*

Significance of Iron-Reducing Bacteria

- Other metals can adsorb to iron oxyhydroxides
- Iron-reducers can solubilize adsorbed metals by iron reduction
- Iron reducers can also solubilize arsenic from minerals such as scorodite

Methods for the Study of Iron-Reducing Bacteria

- Media containing amorphous iron oxyhydroxides
- Molecular techniques

Disadvantages of Present Techniques

- MPN enumeration methods using amorphous iron oxyhydroxides require up to 6 months incubation
- The media currently used are complex and difficult to prepare
- The molecular techniques used do not give quantitative information

OBJECTIVES

- To develop a simple medium for the enumeration of iron-reducing bacteria
- To determine the appropriate carbon source for optimum iron reduction rates
- To test the medium on environmental samples

Media Used in this Study

- Basal salts
- Ferric EDTA
- Different carbon sources were tested
- Two organisms were used to evaluate the media (*Shewanella putrefaciens* 200 (NCIB 12577) and *S. putrefaciens* ESSO 4-1 (NCIB 12580)
- A mixed culture of iron-reducers was also used

Carbon Sources Used

1. Yeast extract + acetate
2. Yeast extract + lactate
3. Yeast extract + butyrate
4. Yeast extract + formate
5. Yeast extract + acetate + lactate + butyrate
6. Yeast extract + peptone (0.5)
7. Yeast extract + peptone (1.5)

Carbon Sources Used

8. Peptone
9. Yeast extract + peptone + formate + acetate + lactate + butyrate
10. Yeast extract + glucose
11. Glucose
12. Yeast extract
13. No carbon source


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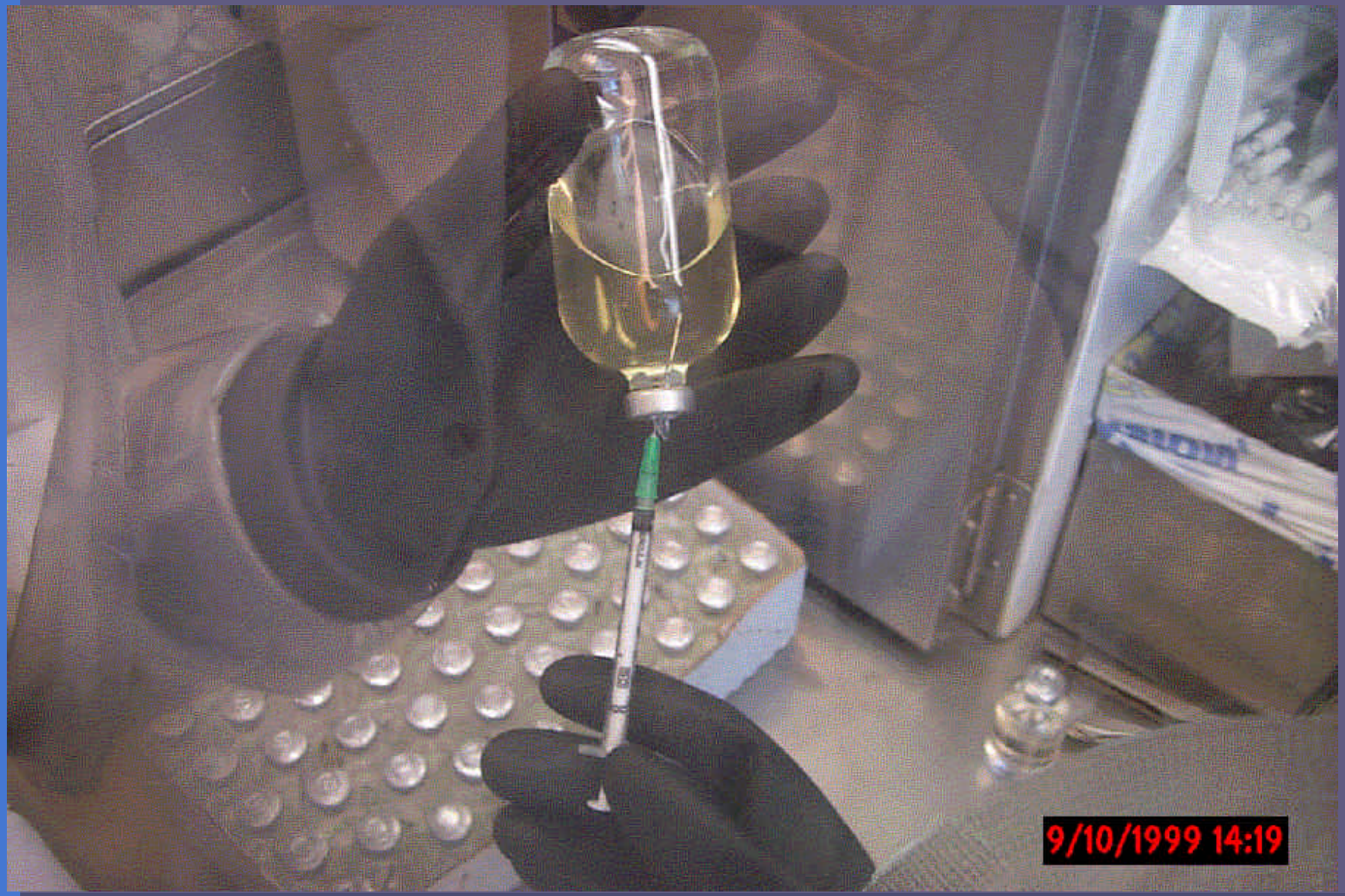
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Ferrozine
Medium 8
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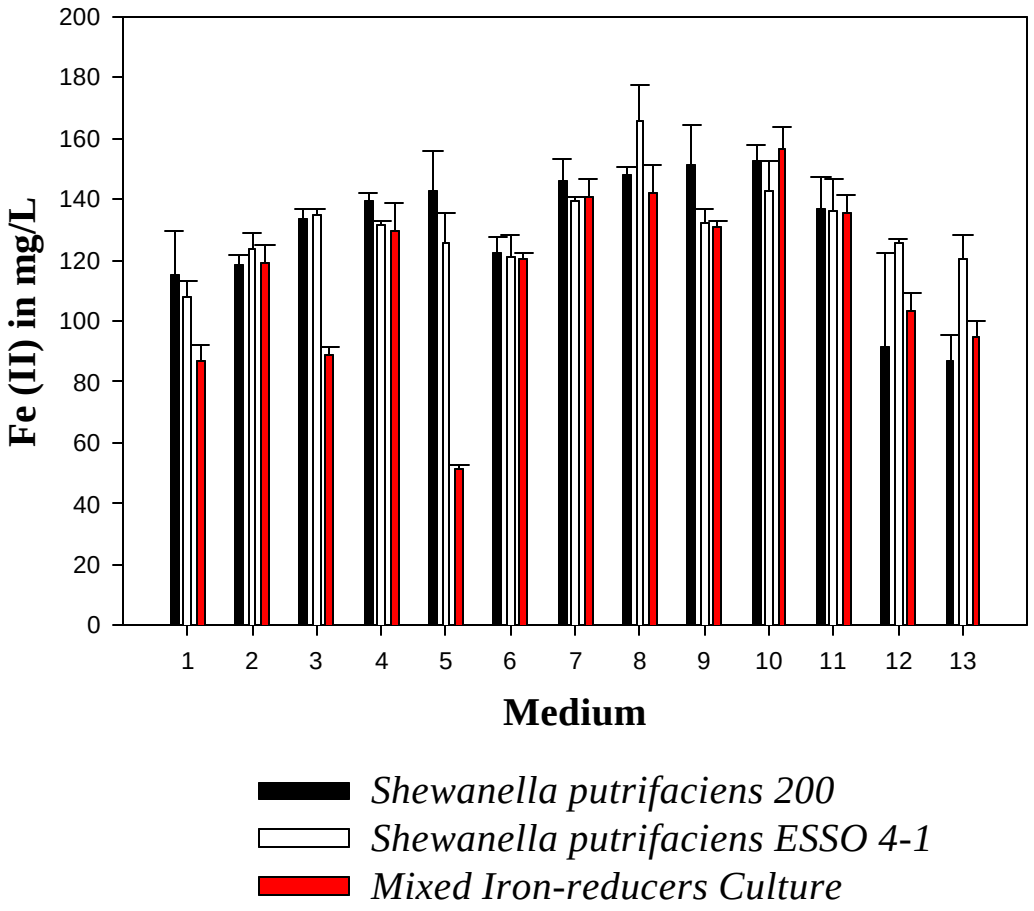
Figure 1. Performance of the three cultures in 13 iron-reducing media after four weeks



Numbers of iron-reducing and heterotrophic bacteria

Depth, (m)	Heterotrophic bacteria (#/g)	Iron-reducing bacteria (#/g)
3.15	1.4×10^4	2.3×10^6
4.33	1.0×10^3	1.7×10^5
4.83	6.1×10^4	7.9×10^3
5.18	9.9×10^4	4.9×10^4
5.45	2.7×10^4	2.3×10^3
5.56	1.2×10^4	4.9×10^3
5.77	1.8×10^4	1.1×10^3
5.96	3.8×10^4	7.9×10^3
6.15	3.3×10^4	2.3×10^3
6.30	2.4×10^5	1.2×10^4

CONCLUSIONS

- A medium was developed for the isolation and the enumeration of iron-reducing bacteria
- The medium is easy to prepare and gives high recoveries
- Results can be obtained in two to four weeks