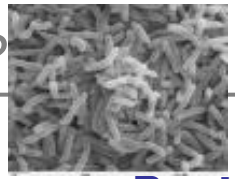
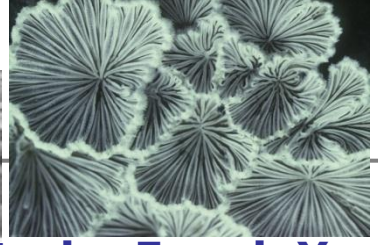
# What are microbes and how did they get in my fuel supply system anyway?

Francesca de Ferra

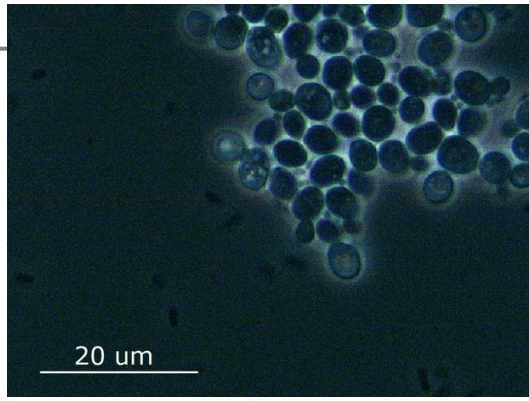
# Who are they?





**Microorganismsms: Bacteria, Fungi, Yeasts all**

**a few microns large cel**


**They require:**

**Water**

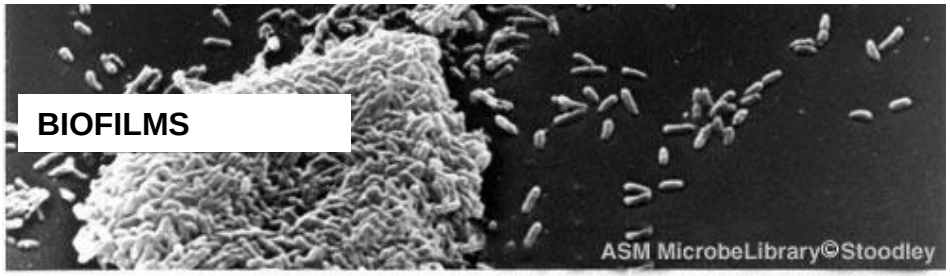
**Temperature**

**C source**

**N, P, S, oligoelements in trace amounts**

**necessary**

**They prefer a structured mode of community life**

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# How did they get there?

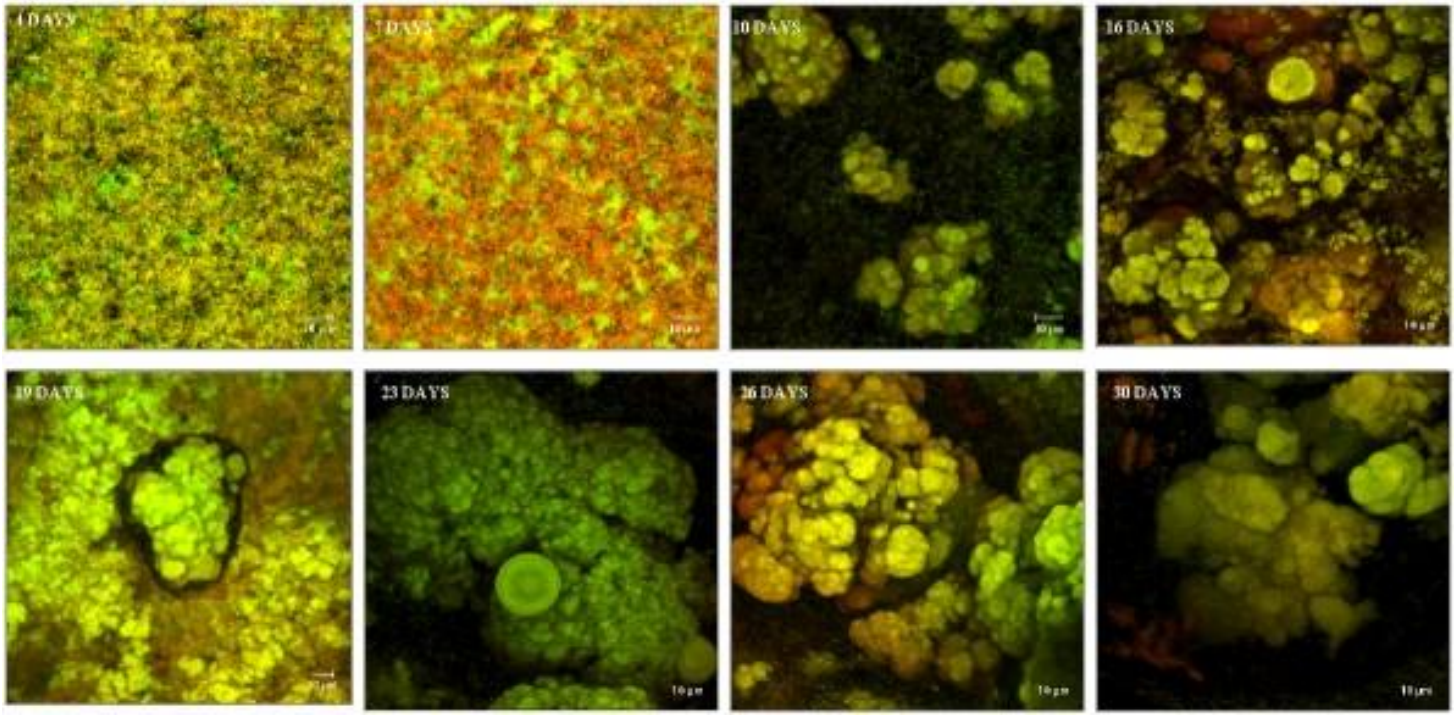
Follow the water…

HydroCarbon (HC) degraders are present everywhere in the environment:

- Soil, rivers, sea water
- Refineries are 'specialized' niches for oil degraders
- Waste water, tanks and deposit water
- Trucks
- Ships etc.

**Even less specialized bacteria are able to degrade methyl esters and fatty acids**

# Biofilms, fouling and corrosion…

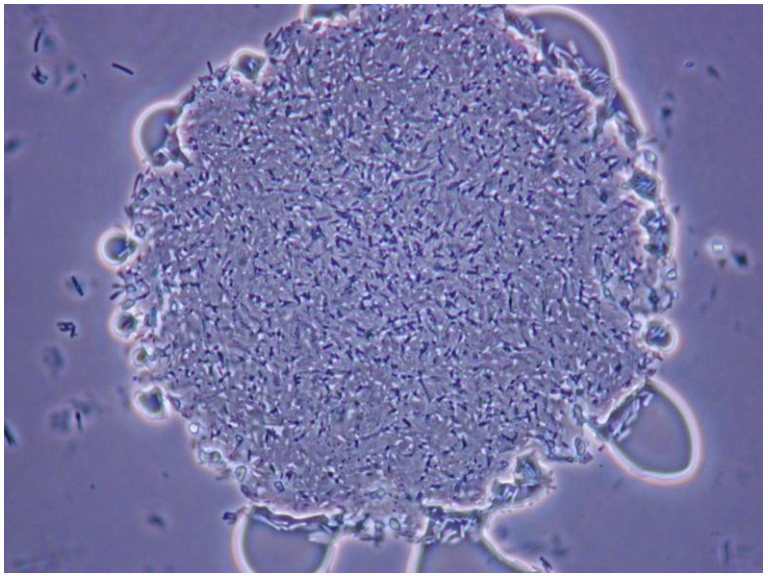



**A microbial biofilm growing with time..**

# Hydrocarbon associated microorganisms

- Solvent resistance
- Oleofilicity
- Production of biosurfactants

- Specific transport systems
- for nutrients



**Aerobic degradation of HC / one major mechanism**

**Anaerobic degradation of HC / diverse mechanisms**

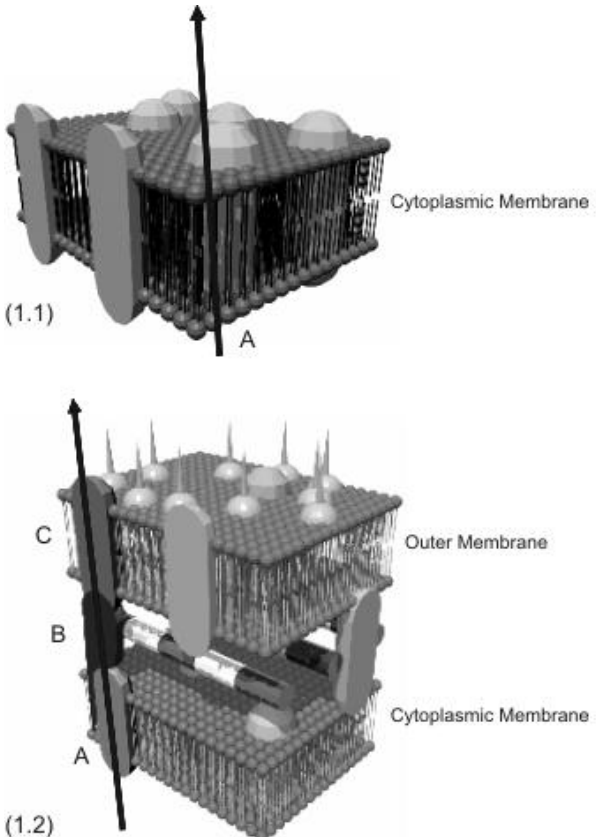
**BIO components stimulate degradation**

***JonathanD.VanHamme, AjaySingh, and Owen P. Ward****

**RecentAdvances in PetroleumMicrobiology**

# Aerobic microorganisms associated with fuel

- Fungi, yeasts and bacteria
- For these microbes degradation needs oxigen
- Mechanism important for oil spills on soils, sea (limit is nutrients N, P)
- $H_2O$ is essential
- Solvent resistance linked to microbial cell wall pumps




**MODELS of CELL WALL SOLVENT PUMPs**

# Findings on anoxic biodiesel biodegradation

- FAME – methyl esters are easily degraded by bacteria in the presence or absence of oxygen.
- Suflita: corrosion is stimulated by diverse inocula from HC degrading communities

- EFFECTS: acid production, biofouling, stimulation of corrosion

**Rich microbial diversity is associated to corrosion**

**Sulfate, nitrate, metal reducing bacteria,**

**Common on metal surfaces**

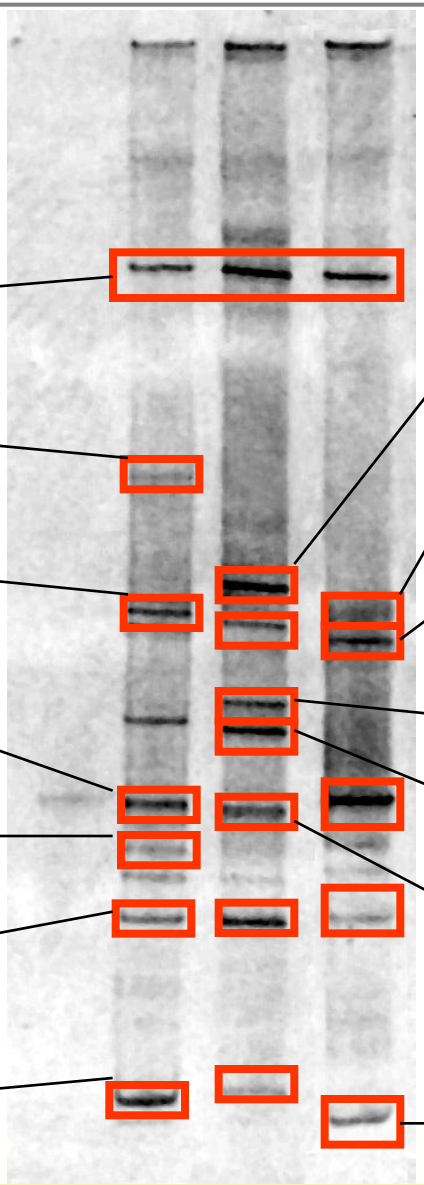
**Very well kown problem in oil production centers**

**ONCE ESTABLISHED BIOFILMS ARE DIFFICULT TO MANAGE**

# Culture independent techniques

**Rich microbial diversity associated to corrosion biofilms:**

## WE know who is there



**Band 1** ***Campylobacter***

**Band 16** ***Petrotoga***

**Band 17** ***Flexistipes***

**Band 7** ***D. Baculatum***

**Band 8** ***Desulfotomaculum***

**Band 12** ***Marinobacter***

**Band 13** ***Fervidobacterium***

**Band 3** ***GreenSulfurBacteria***

**Band 4** ***UAHToil***

**Band 6** ***VP184***

**Band 14** ***PseudoThermotolerans***

**Band 15** ***Marinobacter***

**Band 9** ***Bacteroidetes***

**Band 11** ***UASB TL54***

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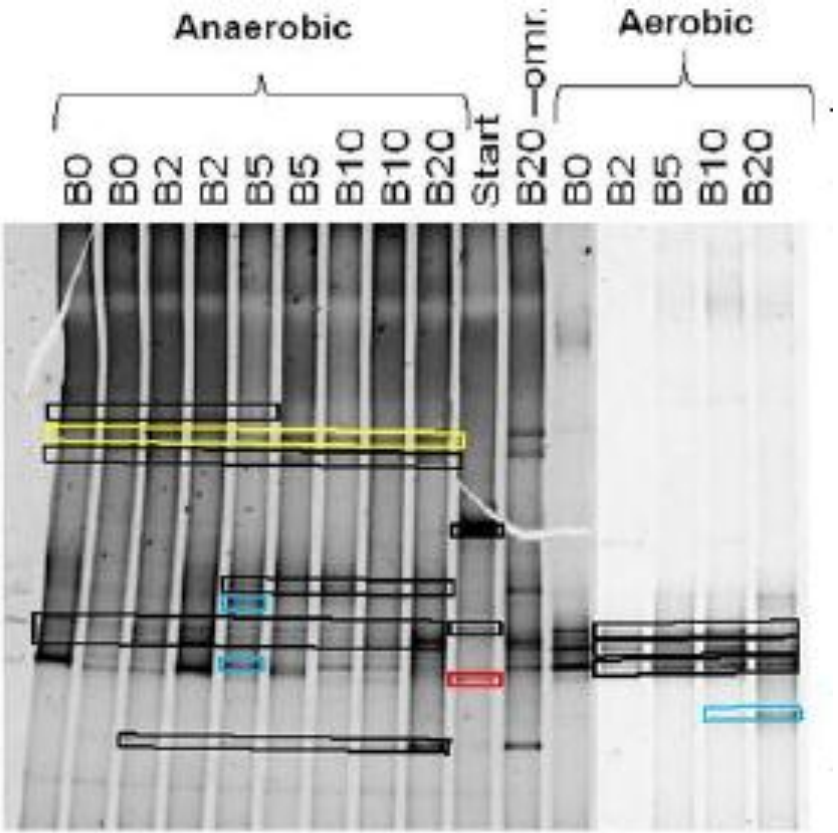
# Culture independent techniques




DGGE gel showing samples with bacteria present as bands in each vertical lane

S Nygaard DTI

**Rich microbial diversity associated to degradation**

**Adding biodiesel to diesel facilitates growth of specific components of bacterial population**

**Every contamination has its own history, with common traits**

# Biocides: mechanisms and adaptation

*'during enhanced oil recovery by water flooding wells are often contaminated with hydrogen sulfide-producing SRBs that result in the souring of sweet crude oils. Biocides have often been found to be ineffective in controlling this problem, while nitrate addition has been used with some success….'*

**Development of resistance to biocides can be due to:**

- **Inactivation of biocides in the fluid surrounding the biofilm**
- **Inefficacy in penetrating the inner strata of biofilms**
- **DNA exchange in parts of the microbe population**

*Microbiology and MolecularBiologyReviews, December2003, p. 503-549, Vol.67, No. 4*

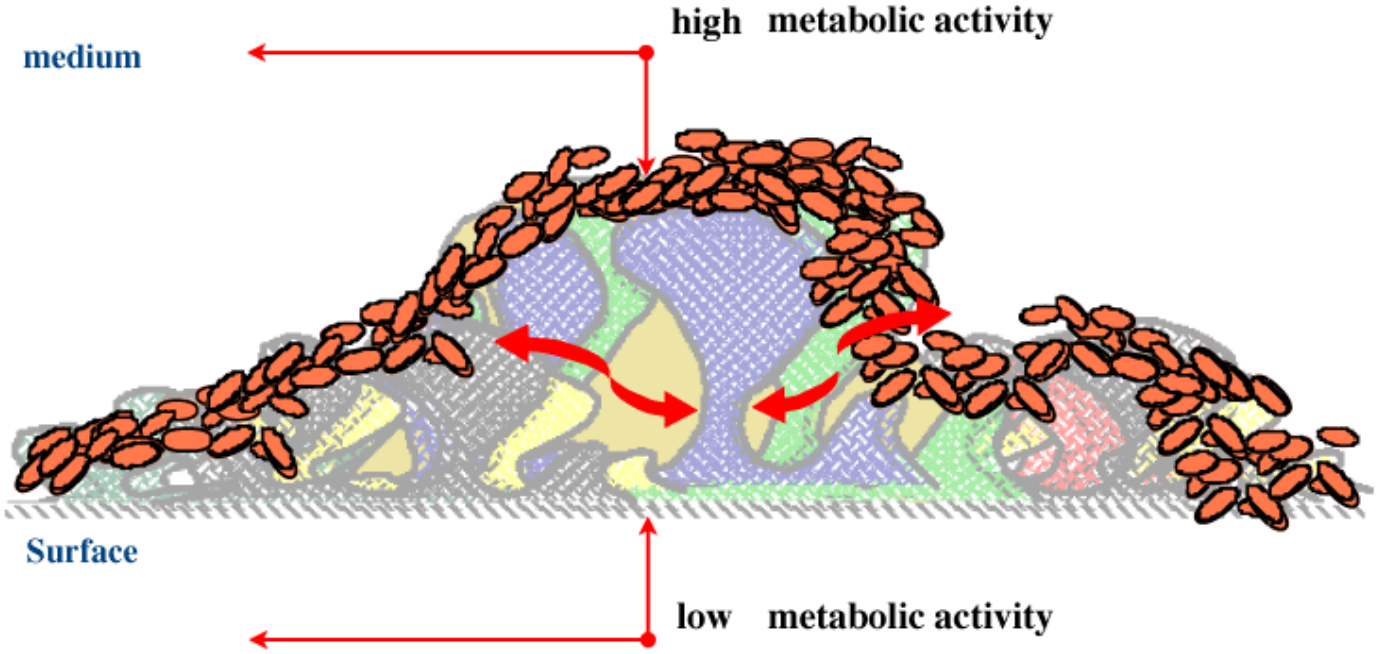
*1092-2172/03/$08.00+0DOI: 10.1128/MMBR.67.4.503-549.2003*

*RecentAdvancesin PetroleumMicrobiology*

*JonathanD.VanHamme,1 AjaySingh,2 and OwenP. Ward3**

# Bacteria need very small quantities of Sulfur

- S is necessary for bacterial life and activity, but very little is needed for survival (ppb)
- Bacteria living in biofilms need minimal quantities



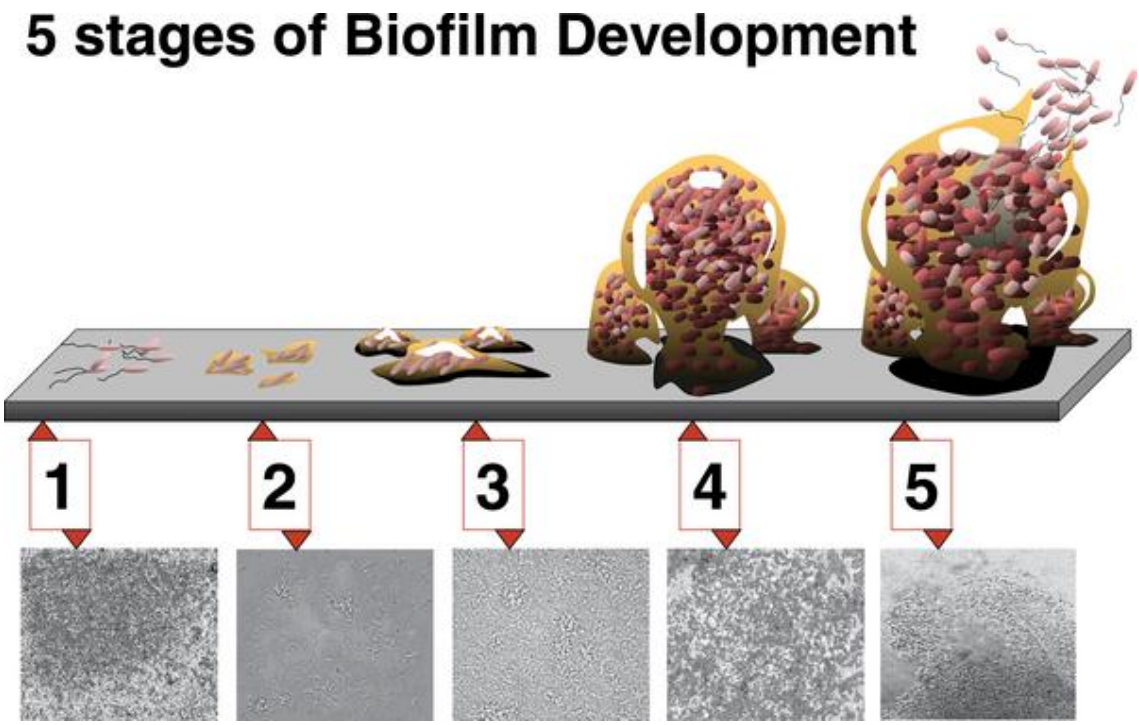
# Toxic sulfur compounds

- **Organo sulfur diesel compnds could be toxic for bacteria growth**
- **DibenzylDisulfide is used in manufacturing corrosion inhibitors**
- **Intermediate compounds of DBT biodesulfurization(sulfones) can have toxic effects on bacterial cells**
- **DBT inhibits *Archeoglobus fulgidus* growth at 10g/l**
- **2OH biphenyl (DBT degradation product) inhibits bacterial growth between 10 and 100 mg/l**
- **DBT tox(shrimp) 0,15-0,5 mg/l /96 hours**

**Lowering organoS concentration increases degradability of diesel**

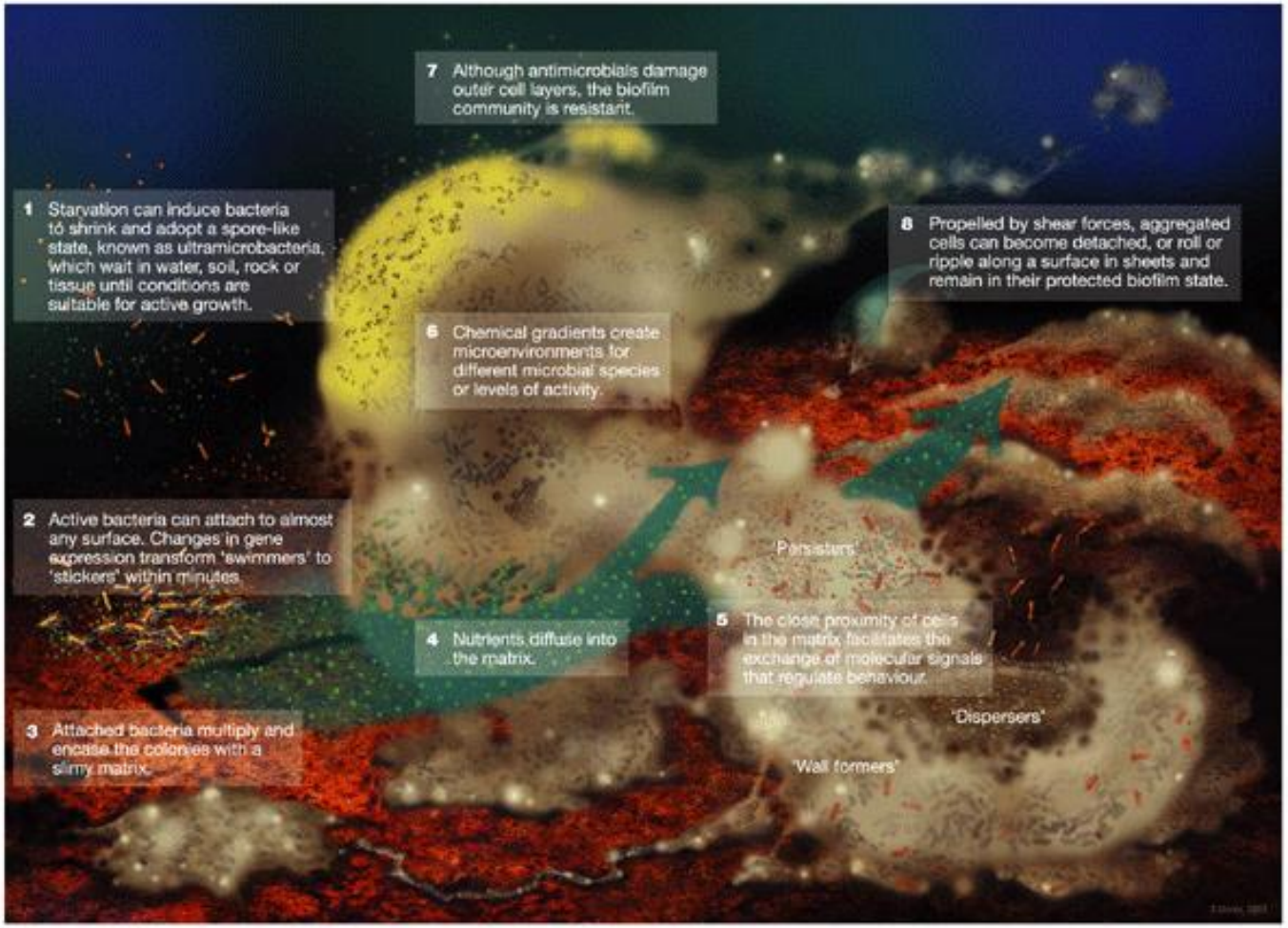
**Addition of Biodiesel further enhances degradability**

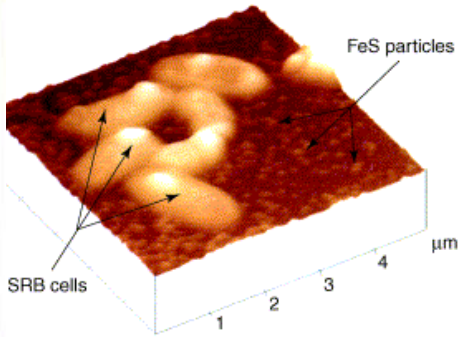
# Biofilm communities protect individual cells

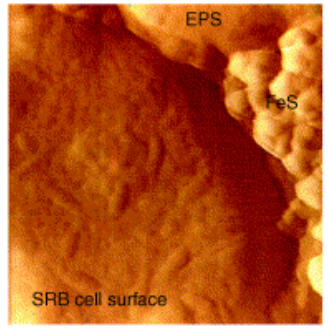



- **Adhesion**
- **Irreversible adhesion**
- **Maturation**
- **Growth and further maturation**
- **Dispersal and colonization**

# Biofilm communities protect individual cells




Current Opinion in Biotechnology

**Bacterial biofilms: from the natural environment toinfectiousdiseases** Hall-Stoodley et al

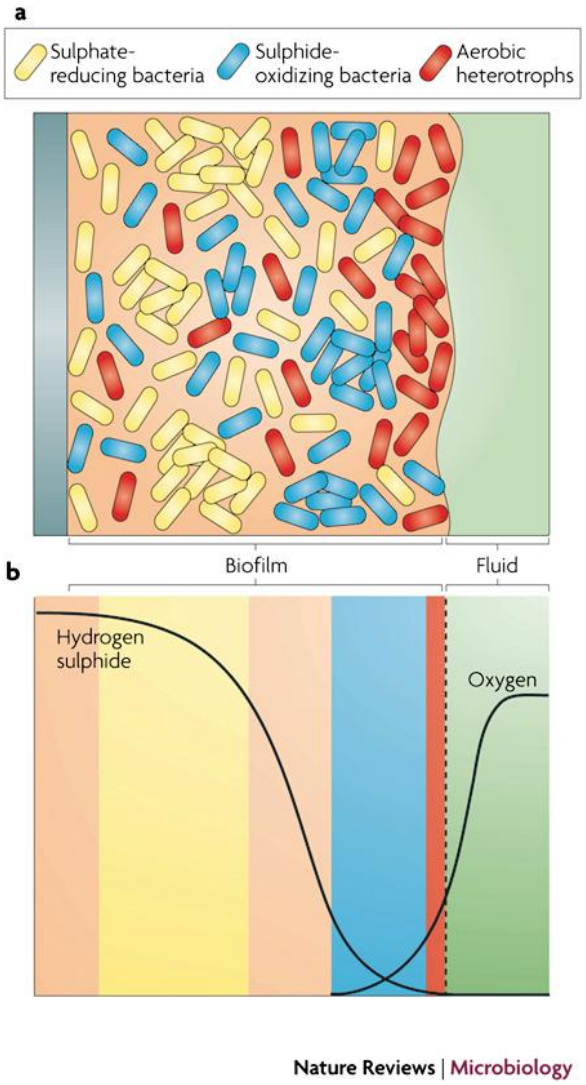
Nature Rev2004

Nature Reviews | Microbiology

**Biocorrosion: towards understanding interactions between biofilms and metals**

Iwona B Beech[1]* and Jan Sunner[2]

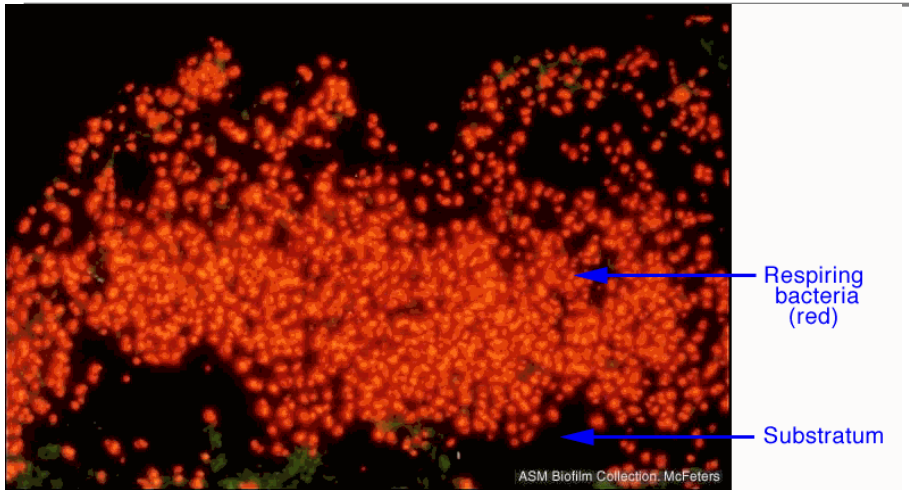
# Microbial diversity in Biofilms

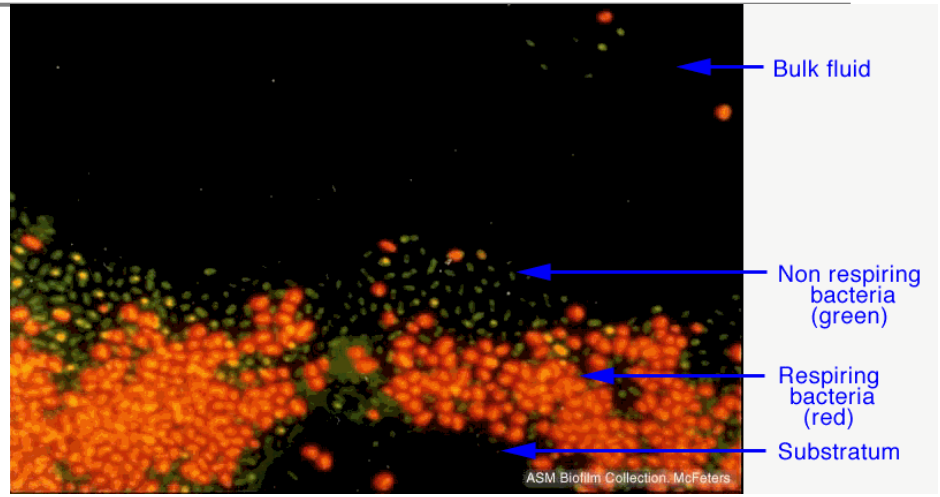


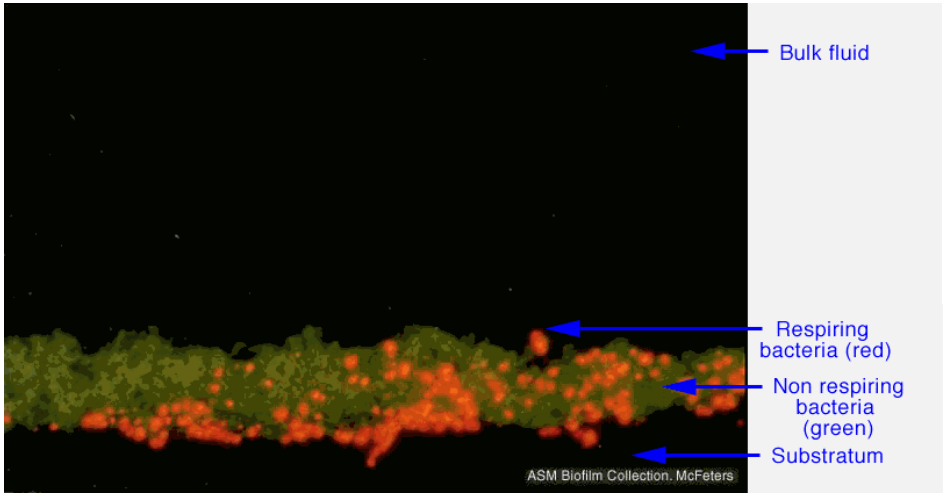

Concentration gradients of compounds (and nutrients) in biofilms are at the basis of heterogeneous microbial populations in biofilms (moulds, bacteria)

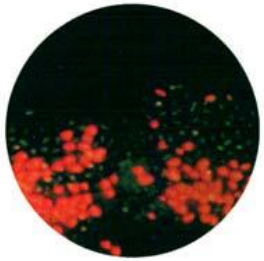
This is also at the basis of complex responses to biocides

# Biocides: mechanisms and adaptation



***After 1 hour exposure, some cells (green) were dead but some, usually deeper in the biofilm, were still alive (red)***

*From Costerton & Stewart - Sci Amer July 2001*

# Conclusions: changes and future management

## What changed?

- Different HC composition, more palatable
- Minor quantity of toxic compnds
- Degradable additives

## What could be done?

- Know your enemy weaknesses
- Do Water analysis, appropriate microbial tests
- Use Cleanness and prevention
- Use biocides sparingly

- Study the integrated system (water cycle, oxygen, ways of entry, environmental factors influencing growth)

Credits to:

The Biotechnology Lab in San Donato Milanese working on Biofuels, Microbiology of Fuel systems and Environmental Microbiology







# And Thank you, questions ?

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