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Institute of Food, Nutrition and Human Health

Control of Biofilms

Associate Professor John D. Brooks

Te Kunenga ki Pūrehuroa

Greetings from New Zealand

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The Problem

Microbiological contamination costs the NZ food industry many millions of dollars annually

Food poisoning alone costs about $100m annually – MAF 1994

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Life As a Bacterium

- The "normal" mode of growth of bacteria is in films on surfaces at an interface
  - May be a few micrometres thick or several mm
  - Modern food processing equipment has many surfaces suitable for colonisation
    - Stainless steel, glass, plastics, rubber, even PTFE
- Conditions in the film are quite different from planktonic (free floating) environment
    - Major portions of genome may switch on or off when cells attach to surface
      - *525 proteins in Alcaligenes change expression over time*

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Definition: Biofilm

An aggregation of microbial cells and their associated extra-cellular polymeric substances, actively attached to, growing and multiplying on a surface

If the organisms are sporeformers, 10% of the film cells may be in the spore form *Sara Scott*

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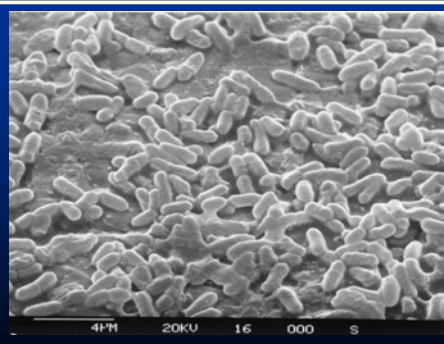
Thermophilic Bacillus Biofilm



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Advantages of Life in a Biofilm

- Concentration of nutrients increased at surface
- Decreased turbulence and scouring
- Diffusion of exoenzymes decreased
- Possibility of organization through signaling
- Transfer of genetic information – resistance genes
- Protection from bulk phase environment
  - Toxins, detergents, sanitizers, antibiotics

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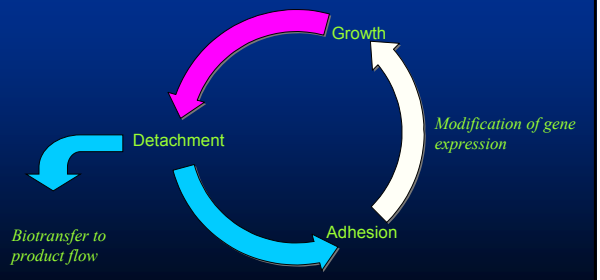
Biofilm Life Cycle



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Microorganisms in the Food Industry

- Increasing evidence of biofilm involvement
  - rapid development in process lines
  - premature shutdown (forced or commanded)
  - increased operational costs
  - reduced profitability
  - interactions of species
    - Increased Listeria attachment and survival with Pseudomonas or Flavobacterium
    - Campylobacter forms films with Enterococcus

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Process Biofilms

- Industrial processes may select populations
- Single species often predominates – (10^7.cm^{-2} after 12 h)
- Growth may be very rapid (t_d = 18-24 min)
- High numbers in product (10^6.mL^{-1})
- Different behaviour from "classical biofilms"

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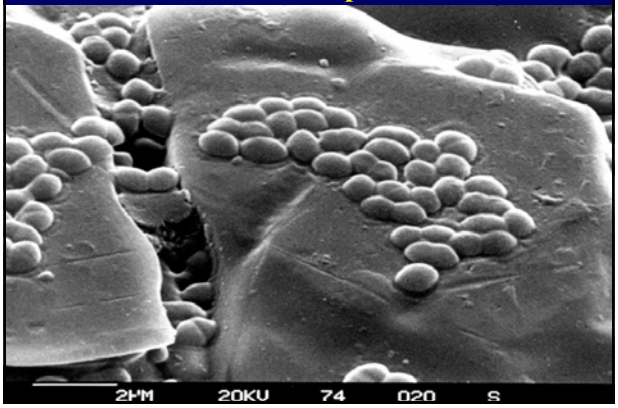
Thermotolerant Streptococcus



Biotransfer – Practical Significance

- Milk evaporator with perfectly clean surface
  - Inflowing milk <<100 thermophiles.mL^{-1}
  - Mean residence time 20-30 mins
  - Outflowing milk up to 10^6.mL^{-1} within 18h
  - *Microorganisms MUST have come from cells immobilised on plant surfaces*

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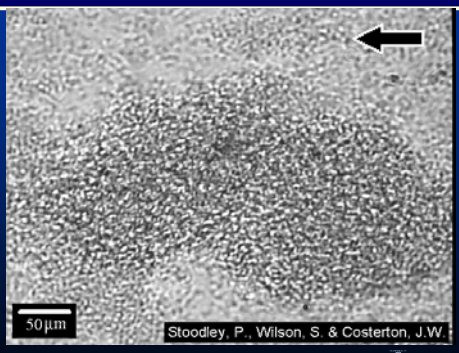
Biofilm in Flow Cell



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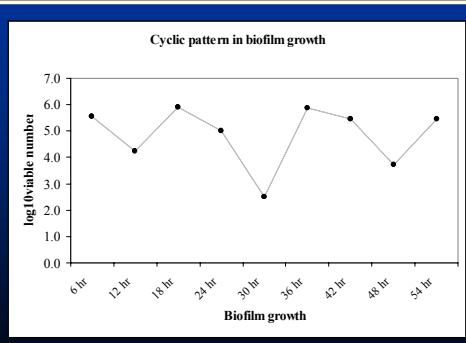
Cyclic Pattern of Growth



Parkar, Flint & Brooks, 2003

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Predictions for Growth

Stage	Separation	Preheat	1st Effect	2nd Effect	3rd Effect	4th Effect	Milk Concentrate
Temp. (°C)	45-55	10-120	69-71	63-66	54-58	45-50	40-58
A_w	~1.00	~1.00	>0.97	>0.97	>0.97	~0.97	0.95
B12-CM	✔	✔	✘	✘	✔	✘	✘
B13-AM	✔	✔	✘	✔	✔	✘	✘
B22	✔	✔	✘	✘	✔	✔	✔
TP13b	✔	✔	✘	✔	✔	✘	✘

Pandeledis, Flint & Brooks, 2001, unpublished

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Removal & Control

- Biofilm cells may be very resistant to cleaning or sanitizing chemicals
- Evidence of greater heat resistance (?)
- Routine CIP cycles may not remove all cells
- Seeding or improved adhesion
  - If cleaning done *properly* total elimination *is* achievable
- Surface roughness affects cleanability
- Use of enzyme cleaners may be more effective than acid or alkali

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Experimental Study

- Bacillus flavothermus from milk powder plant - *Morgan*
- Biofilms generated on cold-rolled 316 stainless steel with 2B finish – standard dairy plant material
- Treated with chemicals by agitation in tubes (lab scale) or under turbulent conditions in a pilot scale cleaning rig (Re>2000)

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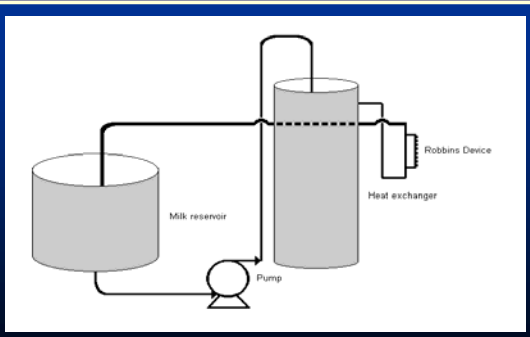
Pilot Plant Cleaning Rig



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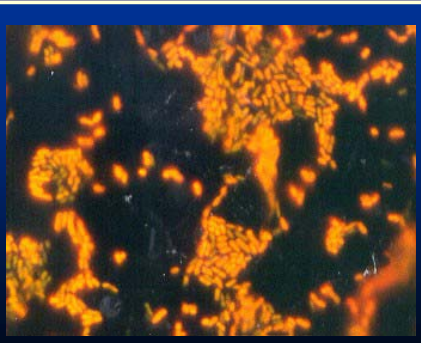
Biofilm on Stainless Steel Coupon



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Standard CIP-treated Film

2% NaOH, 75C, 30 min; 1.8% HNO3 75C, 30 min

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Eroded RJT Joint

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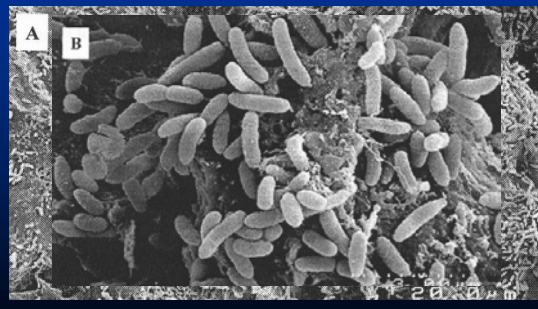
Seal Removed from Plant pre-CIP



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Laboratory Cleaning Trials

Agent	Log Kill	Residue
2% NaOH 75C 30 min 1.8%HNO_3 75C 30 min	7	0
2%NaoH/Na metasilicate 75C 20 min	6	1
Proteolytic enzyme cleaner 60C 30 min	7	0
QAC 25ppm 22C 30min	7	2
Sodium hypochlorite 500ppm 22C 5 min	5.5	2
Chloramine 0.3% 25C 5min	6.8	1
Chlorine dioxide prep 500ppm 25C 5 min	7	2
Peracetic acid/H_2O_2 0.2% 25C 5 min	7	0

In most cases, presence of organic material significantly reduced log kill

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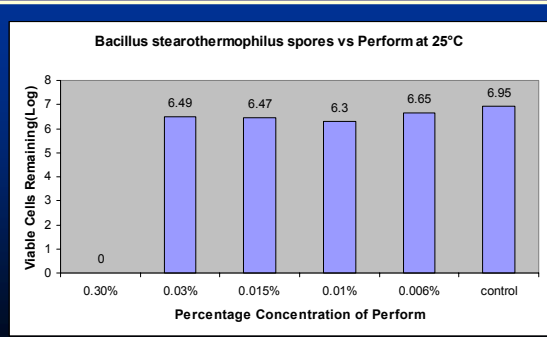
Perform™ Treatment of Spores



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Blocked Evaporator Tubes

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Prospects in Biofilm Control

- Plant surface modification
  - Inhibition of attachment
    - Metalurgy – ion implantation, UGI-clean "Hygienic Stainless"
    - Surface finish – electropolishing
    - Passive coatings
    - Electrical currents
  - Active coatings
    - Bound enzymes e.g. lysozyme

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- Intelligent Plant Operation
  - Minimize area or time at optimum growth temperature
  - Temperature spike or "wave"
- Interference with biofilm organisation
  - Quorum sensing signals
  - Phage attack

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Summary

- Biofilms form within processing equipment
- Proper CIP procedures can remove the films
- Peracetic acid/Hydrogen peroxide sanitizers can kill remaining spores
- Presence of organic matter significantly reduces effect of most sanitizers
- Damaged seals, dead flow areas or remaining fouling can protect bacteria from cleaning agents
- Intelligent operation of plant to deny niche
- Application of new knowledge and technology for control

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