Final Report on the Research Project "Evaluation of Biocides and Disinfection Procedures for the Effective Sanitation of Potato Storages and Equipment"

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Project Sponsors

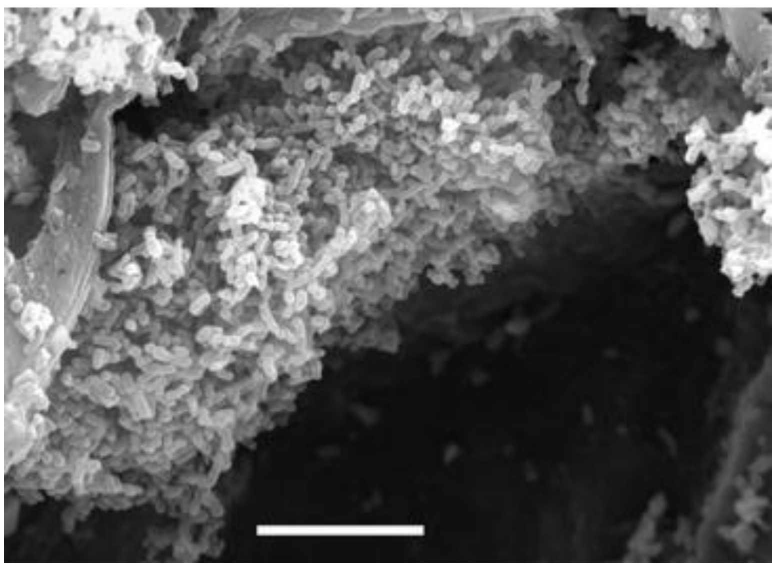
- Canadian Horticultural Council, Seed Potato Sub Committee
- Potato Development Association of British Columbia
- Potato Growers of Alberta
- Alberta Agriculture and Rural Development
- Alberta Crop Industry Development Fund
- Saskatchewan Agricultural Development Fund
- Saskatchewan Seed Potato Growers' Association
- Seed Potato Growers Association of Manitoba
- Manitoba Agri-Food Research and Development Initiative
- Potatoes New Brunswick
- Disinfectant and Equipment Companies
- Growing Forward (Agriculture and Agri-Food Canada)
  - Manitoba Agriculture, Food and Rural Initiatives, Winnipeg, MB
  - Peak of the Market, Carman, MB

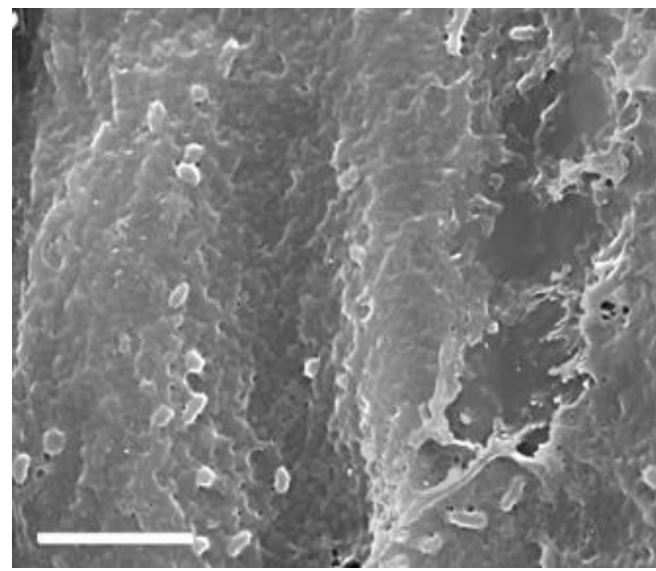
Outline

- Background to the Project
- Study Objectives
- Design and Methodology
- Summary of Results
- Key Recommendations from the Project

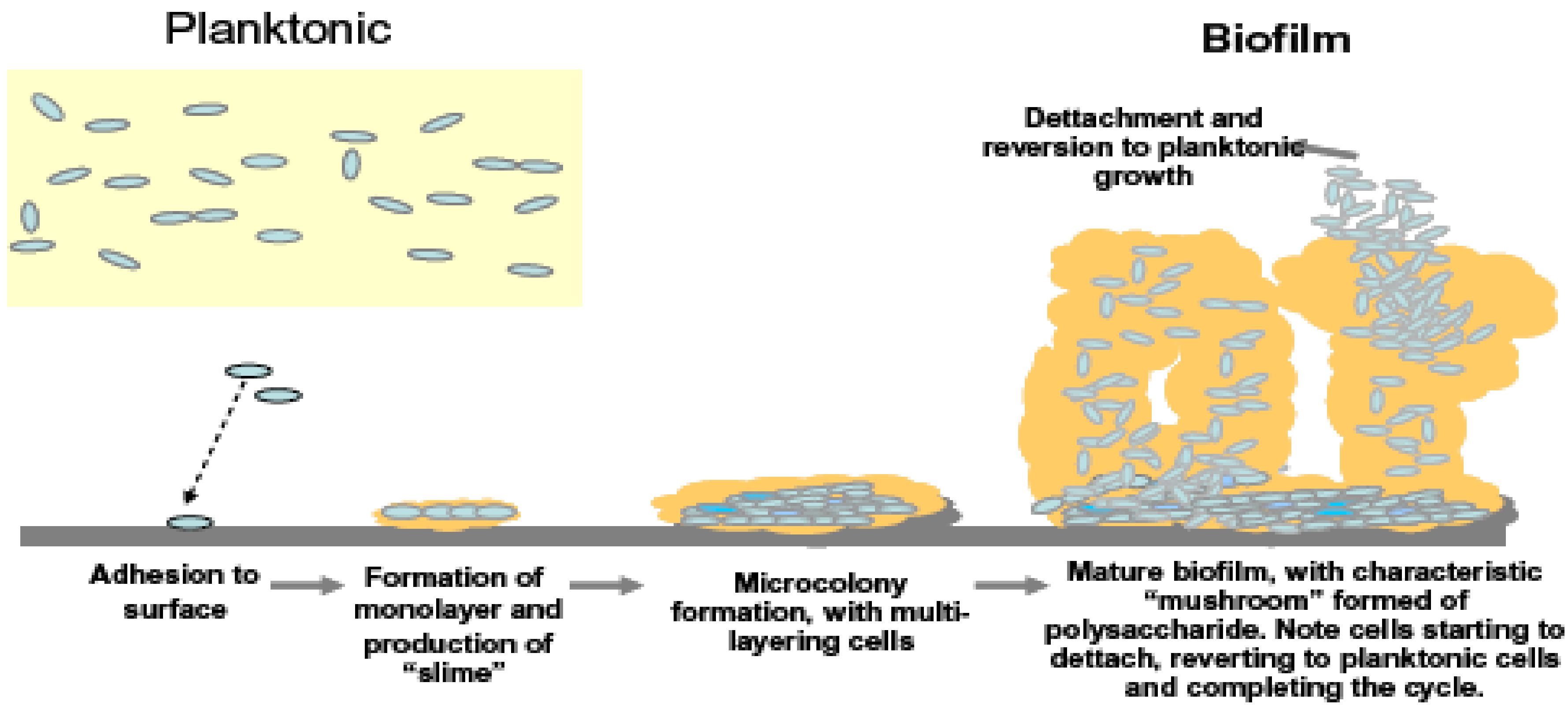
Bacterial Ring Rot of Potato

(*Clavibacter michiganensis* subsp. *sepedonicus*)





Stages of Biofilm Development



Project Objectives

- To identify registered and unregistered chemical disinfectants that are highly effective against *Clavibacter michiganensis* subsp. *sepedonicus*
- To determine the relative effectiveness of these disinfectants against *Cms* biofilms on the various types of hard surfaces typically found in or on potato storages, machinery and equipment
- To assess whether the effectiveness of disinfectants can be improved by the use of detergents, foaming agents, adjuvants and related products

Project Methodology

- The MBEC™ and BEST™ plate assays developed by Innovotech Inc., Edmonton, were the main testing platforms because they facilitated accurate, high-throughput testing of biocides:

  - MBEC = Minimum Biofilm Eradication Concentration

  - BEST = Biofilm Eradication Surface Test
- General protocols followed were the same as were used in previous studies
- Study timeframe: 2008-2011

Project Design

- **Stage 1 -** Determine optimal growth conditions for *Cms*
- **Stage 2 -** Screen 10 commercial and experimental disinfectants against *Cms* biofilms using MBEC™ assay
- **Stage 3 -** Screen 10 disinfectants against *Cms* planktonic cells using the MBEC™ plate assay
- **Stage 4 -** Determine the efficacy of disinfectants against artificial biofilms of *Cms* on 10 types of surfaces using the BEST™ plate assay
- **Stage 5 -** Determine the efficacy of disinfectants against natural and artificial transferred biofilms of *Cms* on 10 types of surfaces using the BEST™ plate assay
- **Stage 6 –** Evaluate the best-performing disinfectants and additives against *Cms* biofilms in pilot-scale trials in commercial potato storages

MBEC™ Assay

MBEC device with 96-peg lid and ridged trough

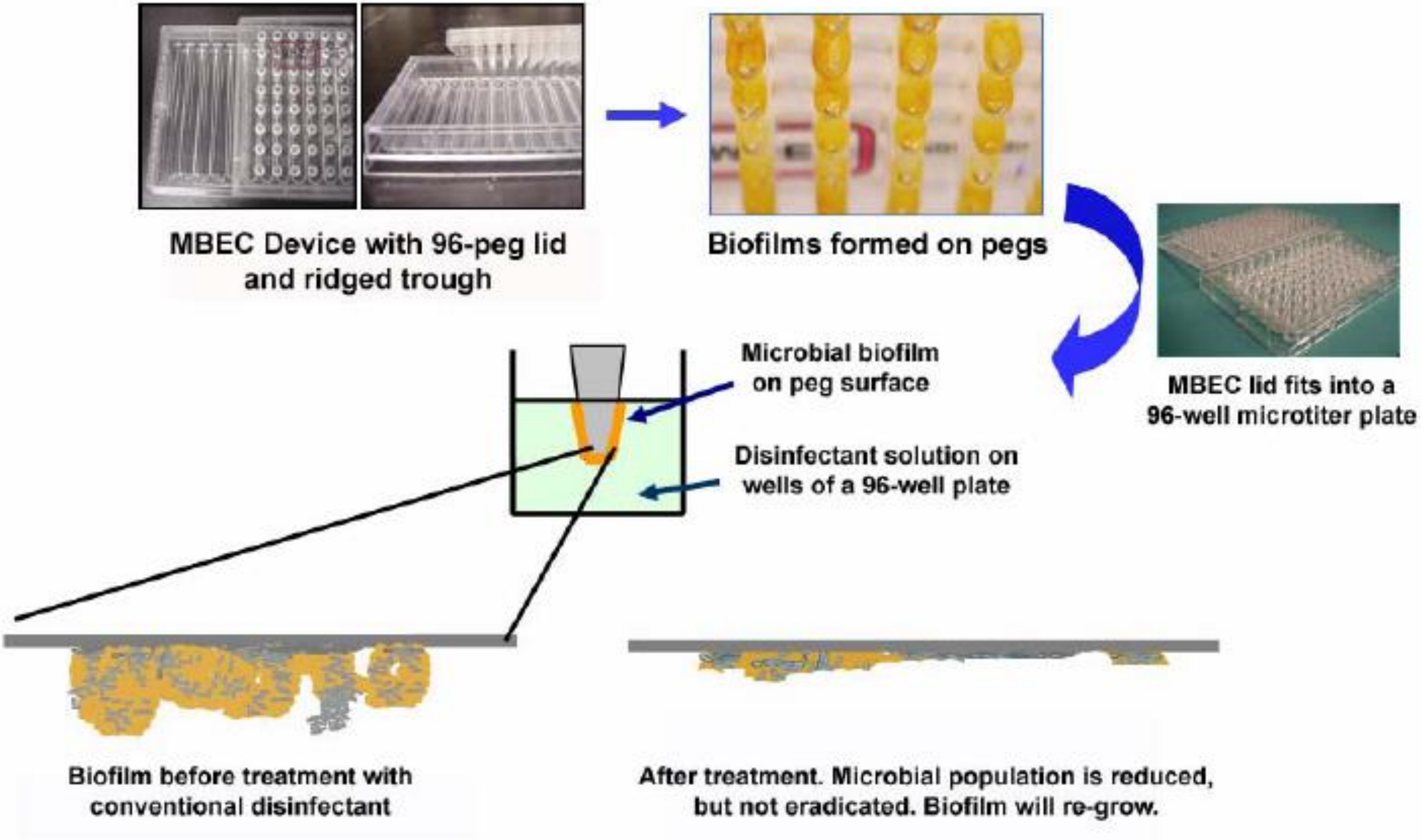
1) Bacterial Inoculum in broth added to trough

2) MBEC placed on a rocker

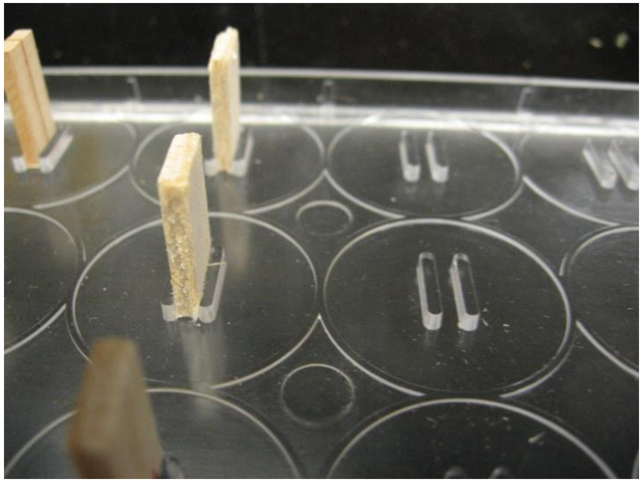
3) Biofilm formation under shear force

4) Biofilms formed on pegs

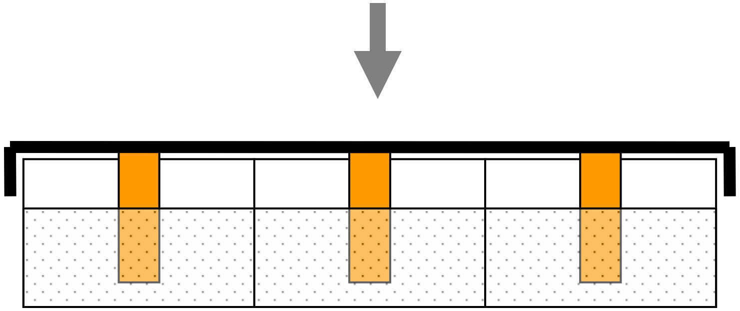
MBEC^{TM} Assay Plate



BEST™ Assay Plate



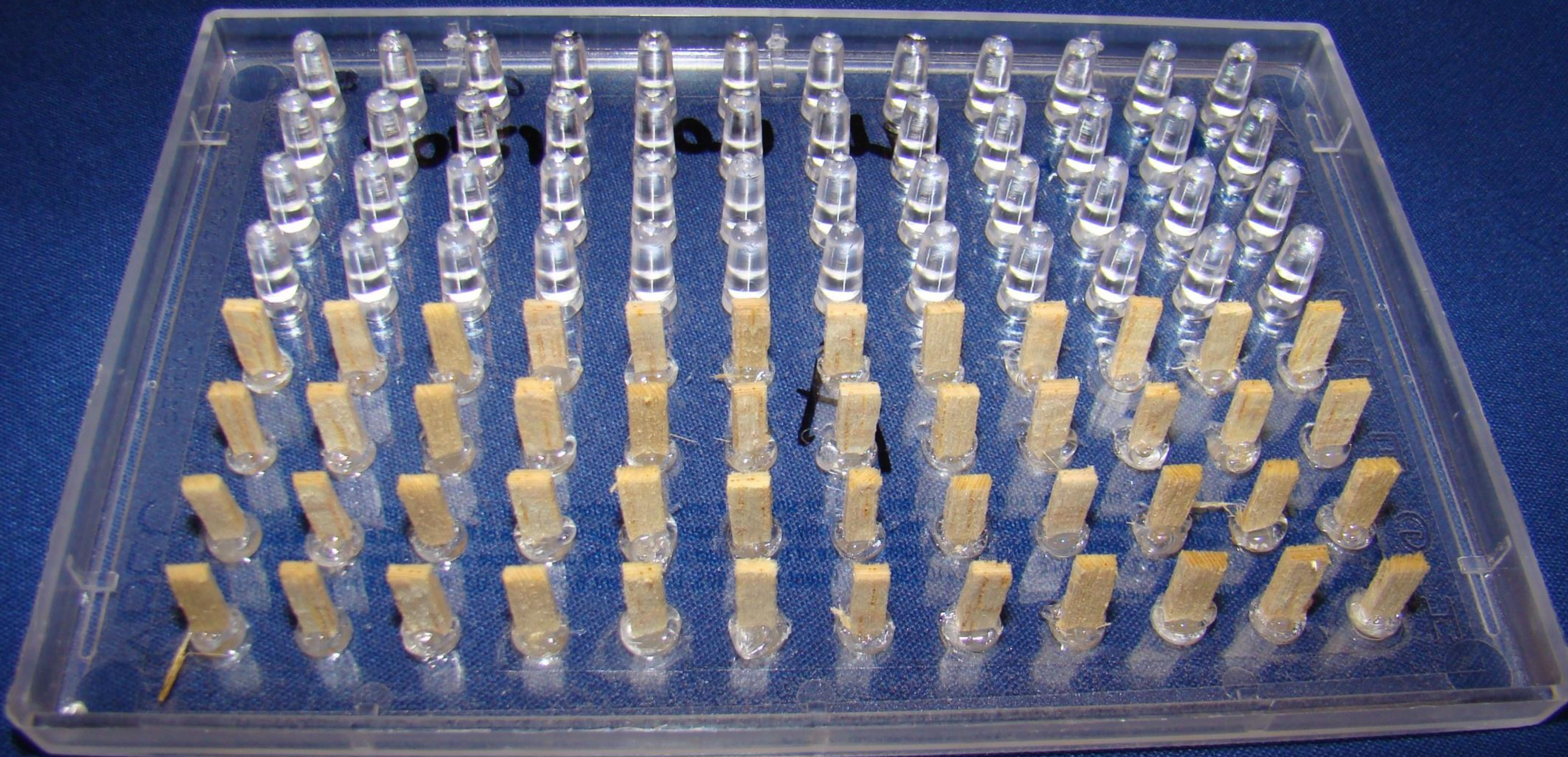
Coupons of hard surface materials are attached to the lid



Stage 1 Methodology
[Optimization of Experimental Protocols]

- Growth times: 3, 5, 7 and 10 days
- Temperatures: 20, 23 and 26°C
- Growth media: Mueller Hinton Broth (MHB), Nutrient Broth (NB) & Yeast Glucose Broth (YGB)
- Platform: MBEC™ assay plate
- Peg materials and coatings:
  - Plastic – Poly-L-lysine- and hydroxyapatite-coated
  - Wood – Balsa, maple dowel and coffee stir stick
- *Cms* isolates: R13, R14 and Cm3s

Modified MBEC™ Assay Plate



Stage 1 Results

- Growth times: 3, 5, 7 and 10 days
- Temperatures: 20, 23 and 26°C
- Growth media: Mueller Hinton Broth (MHB), Nutrient Broth (NB) & Yeast Glucose Broth (YGB)
- MBEC™ plate pegs and coatings:

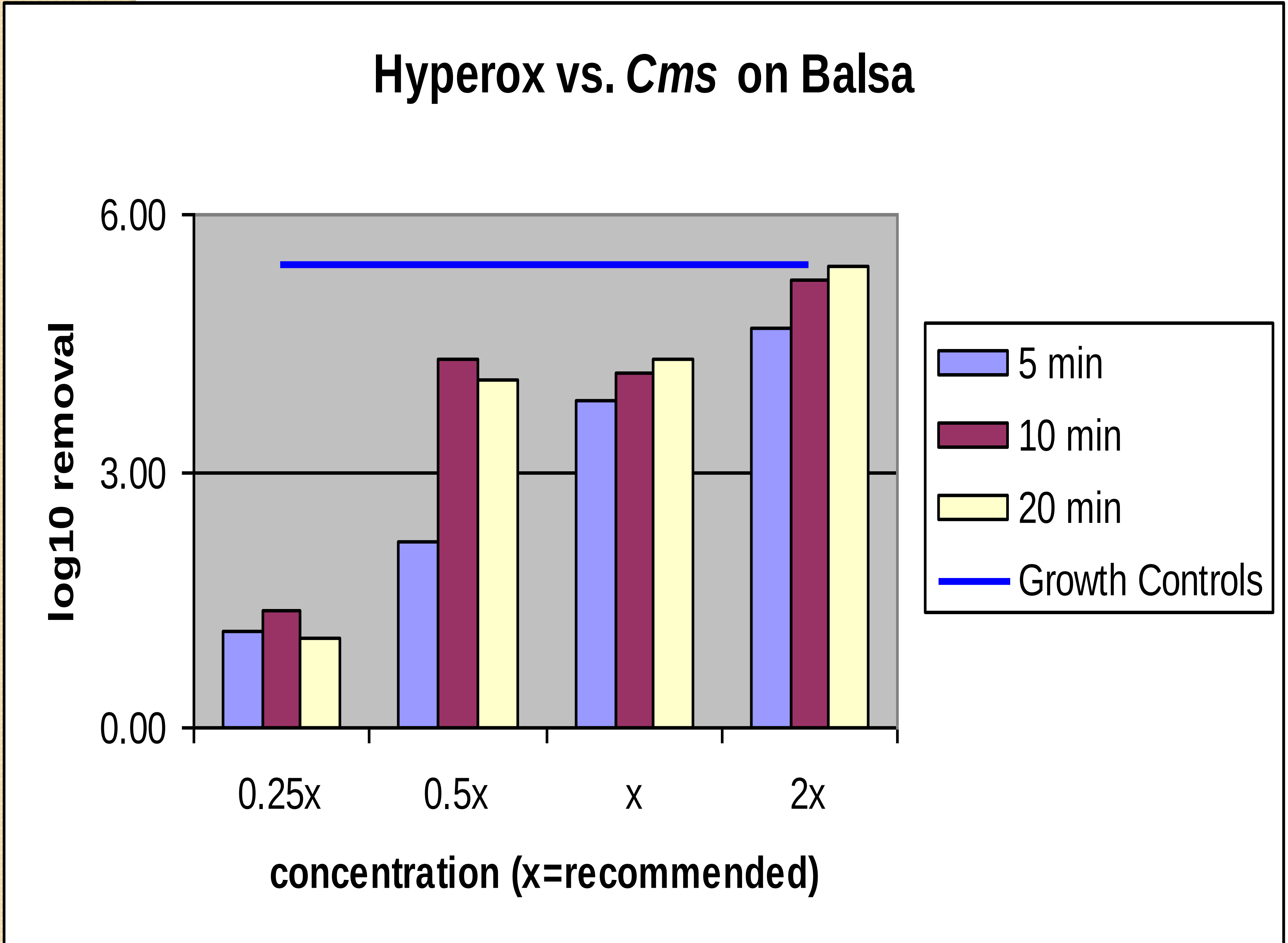
  - Plastic – Poly-L-lysine- and Hydroxyapatite-coated

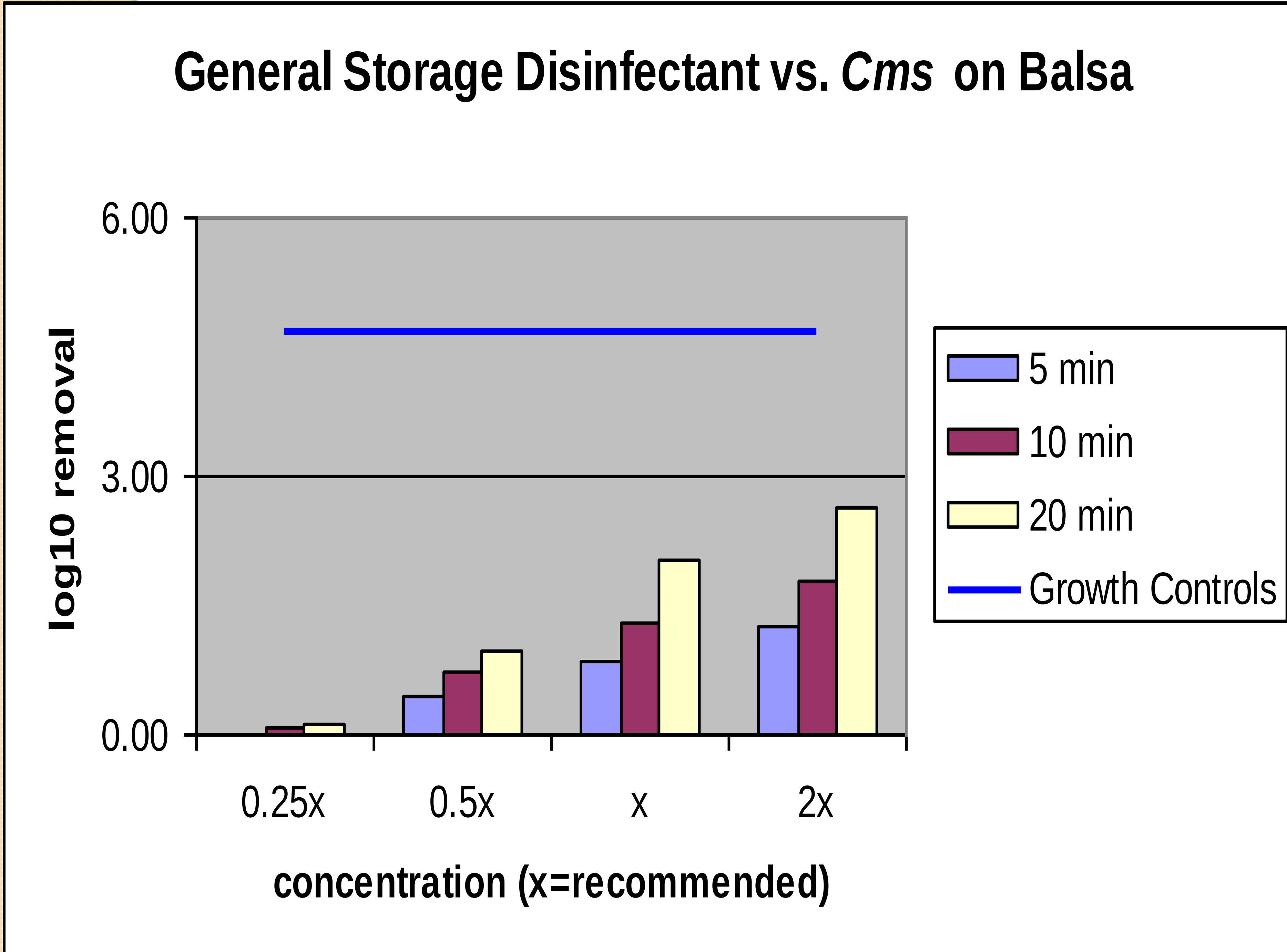
  - Wood – Balsa, maple dowel and stir stick
- *Cms* isolates: R13, R14 and Cm3s

Stage 2 Methodology

[Efficacy of Disinfectants against *Cms* Biofilms]

- Platform: MBEC™ plates with balsa wood and hydroxyapatite-coated plastic pegs
- Disinfectants (10): Bleach, General Storage Disinfectant, SaniDate, Virkon, KleenGrow, Menno Florades, Hyperox, Dutrion, Thymox, and Electolyzed Water (Anostel®/CR-7)
- Concentrations: ¼, ½, 1 and 2X label rates
- Contact Times: 5, 10 and 20 minutes


Hyperox vs. Cms on Balsa
6.00
3.00
0.00
log10 removal
0.25x
0.5x
x
2x
concentration (x=recommended)
5 min
10 min
20 min
Growth Controls

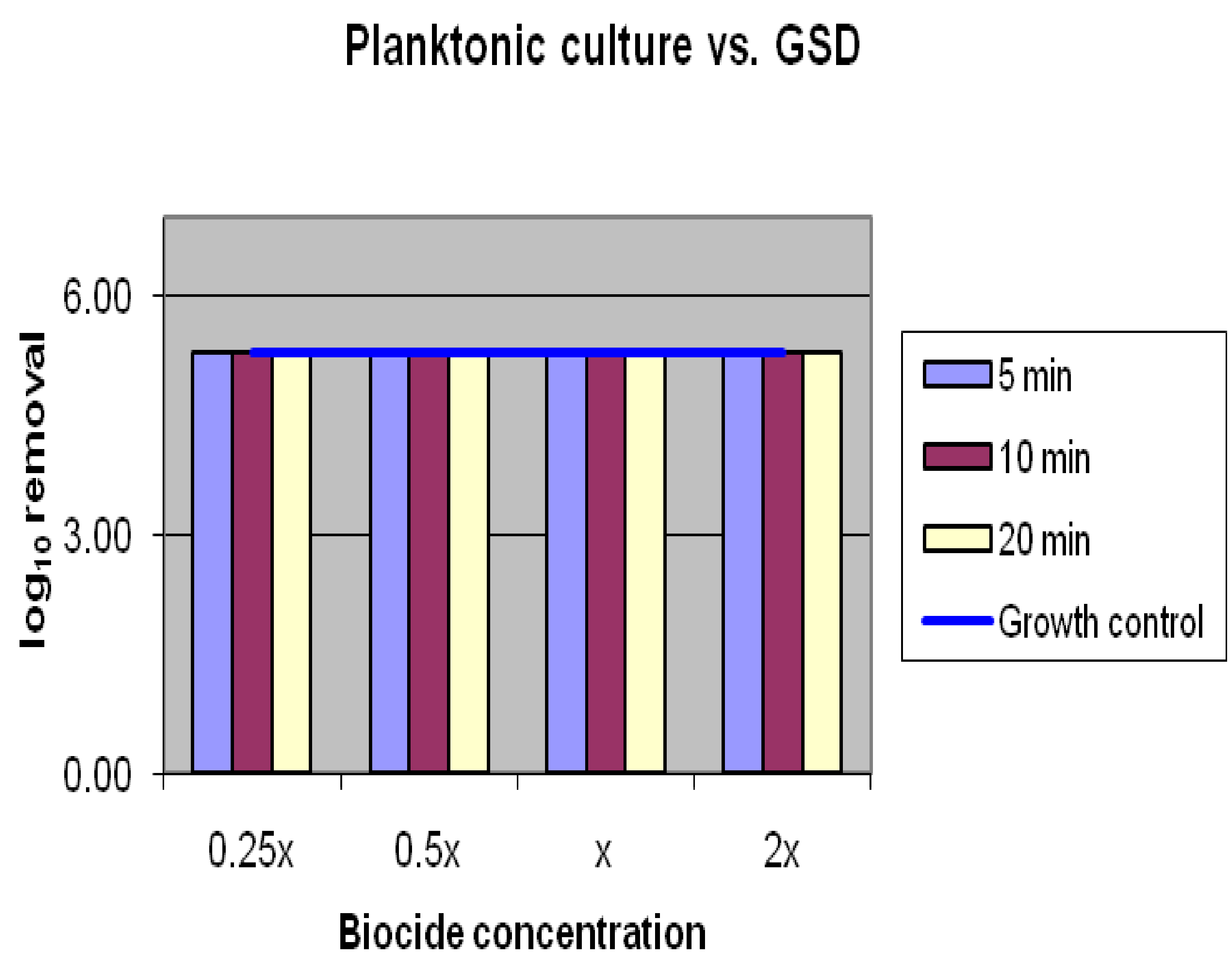

General Storage Disinfectant vs. *Cms* on Balsa
log10 removal
6.00
3.00
0.00
0.25x
0.5x
x
2x
concentration (x=recommended)
5 min
10 min
20 min
Growth Controls

Stage 2 Results

- There was much more biofilm growth on balsa wood pegs versus hydroxyapatite-coated pegs
- The disinfectants were less effective against biofilms grown on wood versus plastic
- Disinfectant efficacy against biofilms improved with increasing concentration and exposure time
- At the 1X rate on balsa pegs, Bleach, Hyperox, Anostel®/CR-7, SaniDate, and Virkon achieved 3-log (99.9%) reductions in *Cms* populations or, in some cases, completely eradicated the pathogen

Stage 3 Methodology
[Efficacy of Disinfectants against *Cms* Planktonic Cells]

- Platform: MBEC™ plates containing 24-hour old cultures of *Cms* grown in Yeast Glucose Broth
- Disinfectants (10): Bleach, General Storage Disinfectant, SaniDate, Virkon, KleenGrow, Menno Florades, Hyperox, Dutrion, Thymox, and Electolyzed Water (Anostel®/CR-7)
- Concentrations: ¼, ½, 1 and 2X label rates
- Contact Times: 5, 10 and 20 minutes



Planktonic culture vs. GSD
log10 removal
6.00
3.00
0.00
0.25x
0.5x
x
2x
Biocide concentration
5 min
10 min
20 min
Growth control

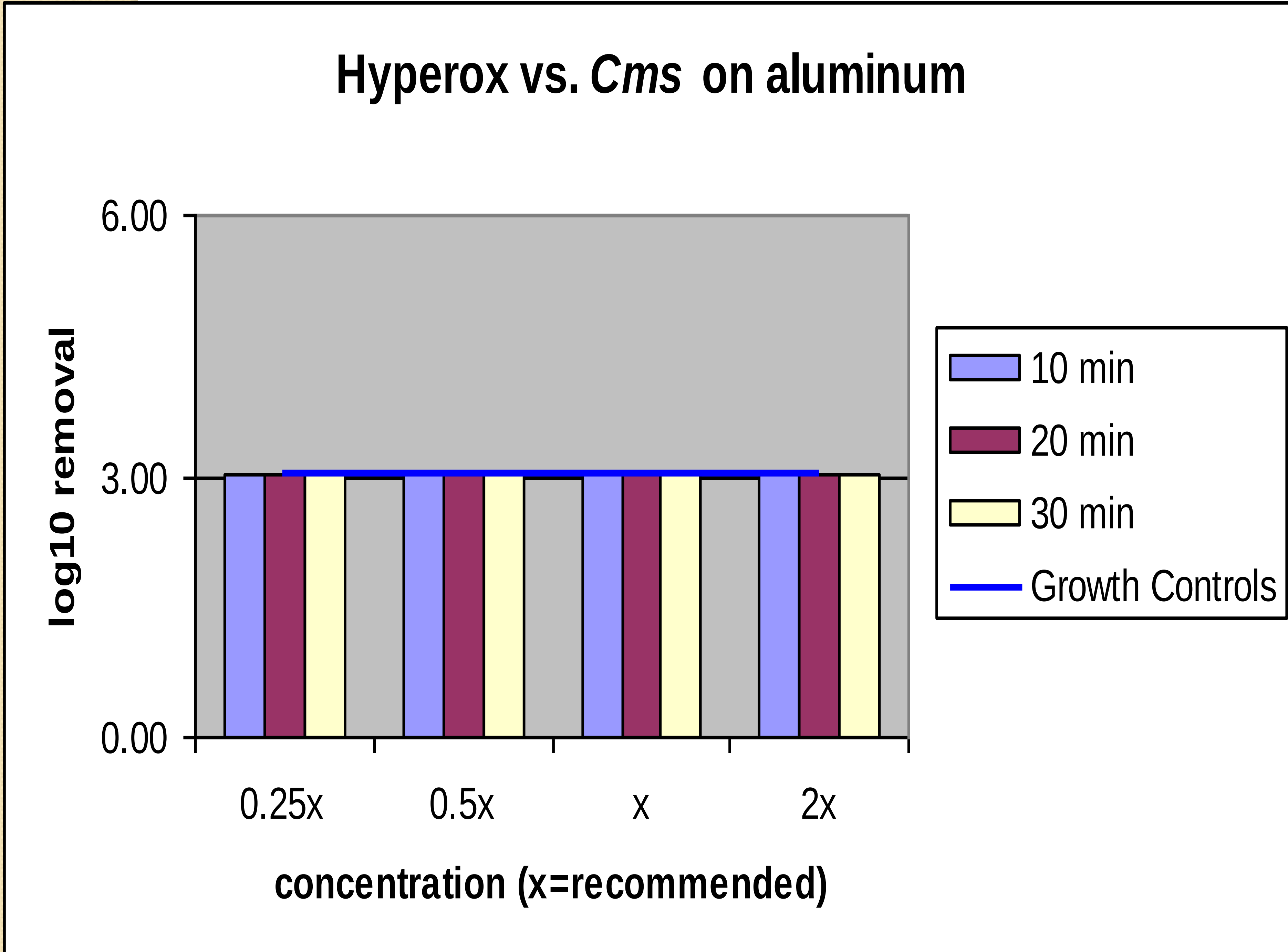
Stage 3 Results

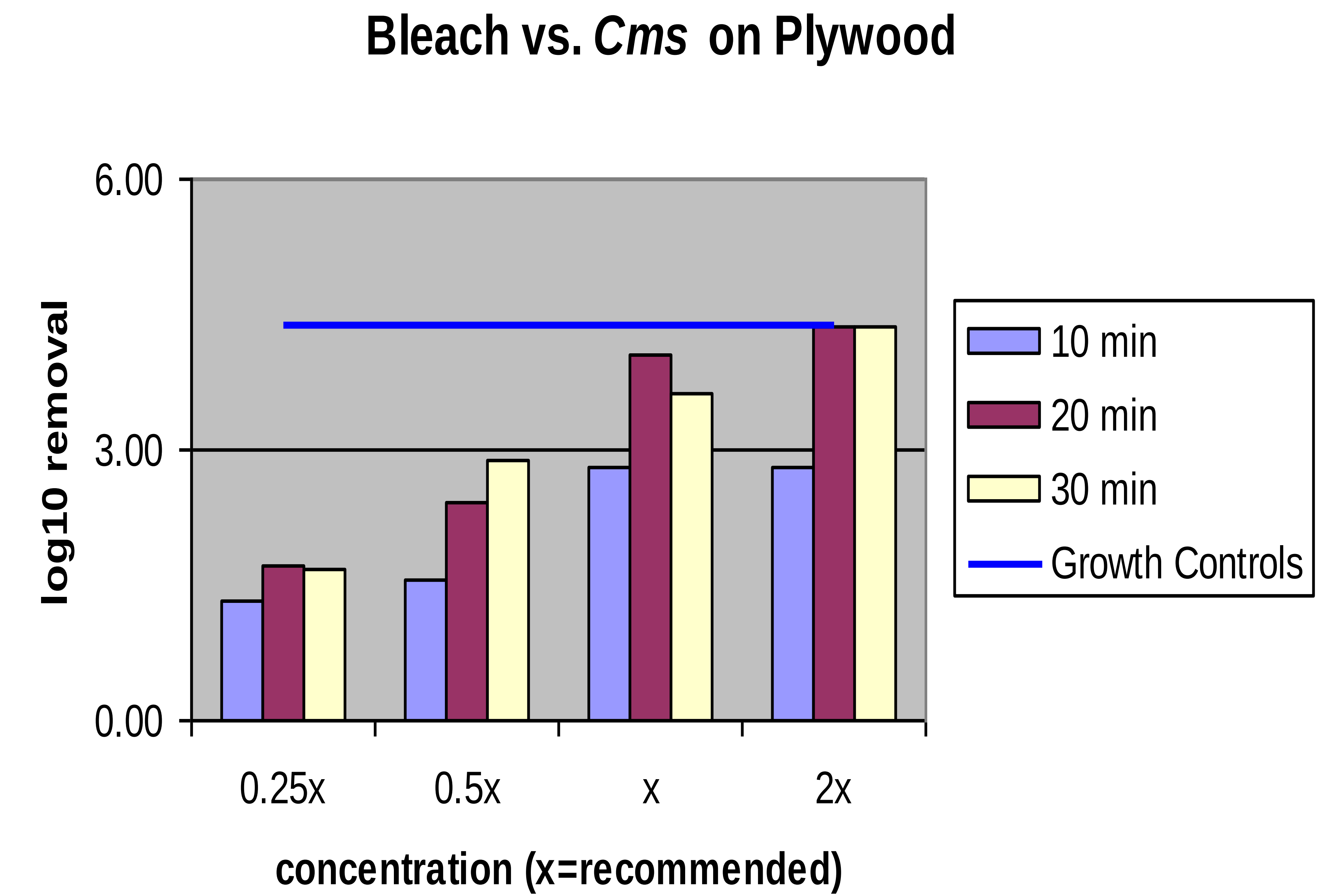
- Bleach, General Storage Disinfectant, SaniDate, Virkon, KleenGrow, Hyperox, and Thymox eradicated *Cms* after as little as 5 minutes of exposure to the ¼X concentration
- Menno Florades required 10 minutes of exposure at the ¼X rate to eradicate *Cms*
- Dutrion required 10 minutes of exposure at 1X to eradicate *Cms*
- Anostel®/CR-7 at ¼X and ½X the label rate was unable to achieve a 3-log reduction

Stage 4 Methodology
[Efficacy of Disinfectants on Infested Surfaces]

- Platform: BEST™ plates (12-wells)
- Disinfectants (10): Bleach, General Storage Disinfectant, SaniDate, Virkon, KleenGrow, Menno Florades, Thymox, Dutrion, Thymox, and Anostel®/CR-7
- Concentrations: ¼, ½, 1 and 2X label rates
- Contact Times: 10, 20 and 30 minutes
- Surfaces (11): Mild steel, stainless steel, aluminum, galvanized tin, polyethylene sheeting, adhesive-backed foam, spray-on foam insulation, rubber belting, high density paper, concrete and plywood

Material	Dimensions (mm)	Source
Mild steel	15 x 6 x 1	Seed Cutter
Stainless steel	15 x 6 x 1	Potato washer
Galvanized steel	15 x 6 x 1	Plenums, wall sheeting
Aluminum	15 x 6 x 1	Ventilation fan
Polyethylene	15 x 5 x (8mil)	Vapor barrier
Foam padding	15 x 5 x 5	Conveyor sides, plenum joints
Spray foam insulation	15 x 5 x 5	Walls, ceilings, truck beds
Rubber	15 x 6 x 5	Belting
High density paper	15 x 6 x (1 layer)	Humidicell material
Plywood	15 x 12 x 4	Wall sheeting
Concrete	15 x 5 x 5	Floors, foundations


Hyperox vs. *Cms* on aluminum
6.00
3.00
0.00
log10 removal
0.25x
0.5x
x
2x
concentration (x=recommended)
10 min
20 min
30 min
Growth Controls



Bleach vs. *Cms* on Plywood
6.00
3.00
0.00
log10 removal
0.25x
0.5x
x
2x
concentration (x=recommended)
10 min
20 min
30 min
Growth Controls

Stage 4 Results

- Poor biofilm formation occurred on steel (3 types), aluminum, polyethylene, and rubber belting
- Porous materials (wood and foam insulation) were much harder to disinfest than non-porous surfaces (steel, plastic, aluminum and rubber)
- The most effective disinfectants against biofilms on plywood and foam insulation were Bleach, SaniDate, Hyperox and Virkon
- Thymox and Menno Florades were partially effective on plywood and foam padding, but not on foam insulation
- Bleach was the most effective biocide overall
- Dutrion, Anostel®/CR-7 and GSD were the least effective biocides

Stage 5 Methodology

[Efficacy of Disinfectants on Transferred Biofilms]

- Platform: BEST^{TM} plates (12-wells)
- Biofilm Sources: Artificial (transferred from 7-day-old YGM plates) and Natural (transferred from BRR infected tubers)
- Biofilm States: Fresh (“wet”) and air dried (“dry”)
- Disinfectants (10): Bleach, GSD, Hyperox, SaniDate, Virkon, KleenGrow, Menno Florades, Dutrion, Thymox and Anostel®/CR-7
- Concentrations: 1 and 2X label rates
- Contact Times: 20 and 30 minutes
- Surfaces: Concrete and wood

Stage 5 Results

- A source of heavily infected BRR tubers could not be found, so this phase of the study was not carried out
- Artificial transferred biofilms were harder to eradicate compared to those grown *in situ* in BEST plates
- The 2X label rate and 30 minute contact time were significantly more effective than 1X and 20 minutes
- Biofilms grown or transferred onto wood coupons were harder to kill than those on cement coupons
- Dry biofilms were harder to eradicate than wet ones
- Top 5 = Bleach, Hyperox, Sanidate, Virkon and Thymox
- Bottom 5 = GSD, Menno Florades, Dutrion, Anostel®/CR-7 and KleenGrow

Phase 6 Methodology

[Pilot-Scale Trials in Commercial Potato Storages]

- Testing Platform: PetriFilm for Bacteria and Yeast & Molds
- Disinfectants (11): Bleach, General Storage Disinfectant, SaniDate, Virkon, KleenGrow, Menno Florades, Thymox, Dutrion, Thymox, Anostel®/CR-7 and 1-Stroke Environ
- Concentrations: Label rate or optimal rate from lab tests
- Contact Times: 20 minutes
- Also tested Wet Steam
- Surfaces: Galvanized steel, concrete, painted metal sheeting, plastic, spray-on foam and aluminum-clad insulation, stainless steel, unpainted wood and plywood

Stage 6 Methodology
[Pilot-Scale Trials in Commercial Potato Storages]

- Storage locations and numbers of bins tested:
  - Alberta (12)
  - Saskatchewan (3)
  - Manitoba (10)
- Status of the bins:
  - None were infested with Bacterial Ring Rot
  - Emptied out but not cleaned (dirty bins)
  - Emptied out and cleaned
  - Emptied out, cleaned and pressure washed
  - Emptied out, cleaned, pressure washed and disinfected
- Microbial assays for bacteria, yeasts and molds (fungi)

Phase 6 Methodology

[Pilot-Scale Trials in Commercial Potato Storages]

- Detergents tested:
  - Carbon-Ate (Hotsy Cleaning Systems)
  - Ripper 1 (Hotsy Cleaning Systems)
  - Ripper 2 (Hotsy Cleaning Systems)
  - Super XLT (Aaladin Superior Cleaning Products)
  - 1-Stroke Environ (Steris)
  - General Storage Disinfectant (Ag-Services Inc.)
- Applied with a low pressure nozzle
- Surface dwell time = 10-20 minutes
- Residual detergent was pressure washed away
- Sampled before detergent and after pressure washing

Mobile Sanitation Unit

Hotsy Pressure Washer/Steamer

MSU Water Tank and Hoses

Commercial Potato Storage, Taber, AB

Preparing to Apply Disinfectants

Typical Surfaces in a Storage

Cleaning with Wet Steam

Applying a Disinfectant

Swabbing a Treated Surface

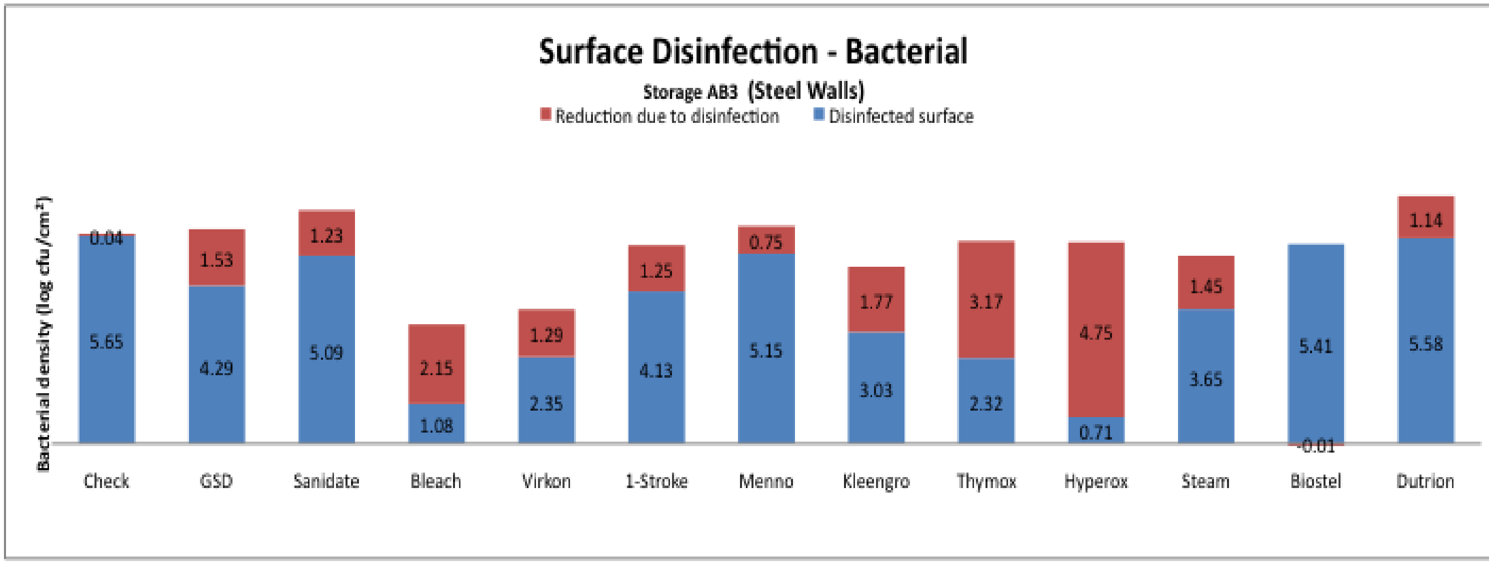
General Observations

- Storage and equipment sanitization programs are being widely used by potato producers and processors in Western Canada
  - Best management practice (disease control)
  - On-farm food safety or biosecurity programs
- Three key steps are involved:
  - Rough cleaning to remove tubers, vines, soil and dust
  - Pressure washing or hosing down
  - Disinfectant application
- Sometimes, two of these steps are combined

General Observations

- Microbial sampling is not being routinely used as a way to assess the effectiveness of sanitization programs in storages and on equipment
- Detergents are rarely being used during the pressure washing stage
- GSD is the most popular disinfectant and is sometimes used as a cleaner-disinfectant during pressure washing
- Most growers and storage managers are not aware of the availability of other registered disinfectants for BRR control

Stage 6 Results
[Pilot-Scale Trials in Commercial Potato Storages]



Stage 6 Results (Taber, AB)

Surface	Bacteria (3-log reduction)	Fungi (3-log reduction)
Concrete floor	Bleach Hyperox	Bleach
Spray-on foam insulation	Bleach Anostel®/CR-7	Bleach
Galvanized steel walls	Hyperox Thymox	Bleach Thymox
Galvanized steel plenums	None	None

Stage 6 Results

[Pilot-Scale Trials in Commercial Potato Storages]

- Bacteria were more difficult to eradicate compared to yeasts and molds
- 1-Stroke Environ was the best-performing detergent
- Bleach achieved a 3-log reduction in microbial numbers more than any other treatment tested
- Performance of other disinfectants was variable and depended on the kinds of microorganisms being targeted and type of surface being treated
- Wet steam was generally less effective compared to the chemical disinfectants

Key Recommendations

- Three key steps need to be followed for effective sanitization of potato storages and equipment:
  - Rough cleaning
  - Pressure washing, hosing down or compressed air
  - Application of a registered disinfectant
- Select disinfectants based on disease history, storage features, ease of use and potential risks
- Rotate the types of detergents and disinfectants being used to sanitize potato storages to minimize the risk of resistance developing in pathogens and other microbial contaminants

Key Recommendations (Continued)

- Most disinfectants are biocidal and are effective against bacteria, fungi and yeasts
- Three disinfectants are registered in Canada that specifically mention Bacterial Ring Rot and/or *Cms* on the label:
  - General Storage Disinfectant
  - SaniDate Disinfectant
  - 1-Stroke Environ Germicidal Detergent
- Equipment, machinery and storage sanitization should be an integral part of an overall potato disease management (biosecurity) program

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