A Review of Rapid Microbiological Analysis of Yoghurts and Fermented Products for Detection and Enumeration of Spoilage and Indicator Organisms

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Content - agenda

- Spoilage and Indicator organisms and why we test for them?
- Yoghurt and fermented products
- Rapid Alternative Methods
- Brief history of automation
- Challenges to adopting alternative methods
- Summary and thoughts

Microbiological Testing

Food Safety
Pathogens
Detection Presence/Absence
PRTOTECTION OF THE CONSUMER FROM EXPOSURE TO HEALTH HAZARD

Quality Indicator Testing
Quality indicators for food products or processes
ENSURING PRODUCT DOES NOT SUFFER MICROBIAL DETERIORATION DURING SHELF LIFE

Hygiene Quality
Indicators of potential hazards
(e.g. Entero/coliform)
HACCP Risks

Why Test for Spoilage and Indicator

- Product is safe for release and achieve stated shelf life.
- Validation and verification of HACCP.
- Monitoring variation within and between products
- Surveillance of a supplier performance
- Provision of due diligence data
- Monitoring the environment, equipment and personnel
- Data to demonstrate compliance with legal criteria or accepted guidelines
- Compliance with customer specifications

Fermented Milk Products

- Fermentations involving production of lactic acid - origins lost in antiquity.
- Middle East and East Mediterranian.
- Improved keeping qualities.
- Acquired taste .
- Skills in making new fermented milk products
- Metchnikoff (1910) stirred interest suggesting health improving benefits- longevity in Bulgarian hill tribesmen
- 1950’s introduction of fruit and flavoured varieties of yoghurt

Fermented Milk Products

- Lactic Fermentations
  - Mesophilic (20c) e.g. Cultured Buttermilk
  - **Thermophilic (37-45c) – e.g. yoghurt**
  - Therapeutic – e.g. Yakult
- Yeast-Lactic Fermentations
  - E.g. Kefir, Kumiss, Acidophilus-yeast milk
- Mould Lactic Fermentations
  - E.g. Villi

Yoghurts

- Yoghurt: (Str. thermophilus, Lactobacillus)
  - full, medium, low fat
  - Method of production- Set or stirred
  - Flavoured (natural fruit/flavourings)
  - Processed: freezing, drying, concentrating
- 'Yakult' (Lactobacillus casei)
- Bio-Yoghurts (Str.thermophilus/Lactobacillus acidophilus, Bifidobacterium bifidum)

Microbiology

- In general terms, although not to be complacent, yogurt can be regarded as hygienically safe.
- Acidity, low pH (~1% Lactic Acid): therefore pathogens like Salmonella are largely inactive.
- Coliforms unable to survive low pH, reinforced by potential production of antibiotic substances produced by the yoghurt fermentative organisms.
  Typical spec <10 cfu/g

Microbiology

- YEAST AND MOULDS:
  - little affect from pH
  - Sucrose and lactose available as energy sources
- Yeasts are of particular concern
  - Domers
  - Lactose utilising - build up on surfaces
  - Yeasts from fruit and fruit purees (e.g. Saccharomyces cerevisiae).

Yeast and Moulds

- YEASTS: Doming – of aluminium caps- common indication of yeast activity.
- Specs:
  - Target Figure: <10 cfu/g (y&m)
  - Acceptable: 10-50 cfu/g
  - Acceptable but to be improved on:50-100 cfu/g
  - Unacceptable: 100 cfu/g
- MOULDS: tend to be less of a problem as the moulds can only grow satisfactorily at the yoghurt/air interface, agitation tends to suppress development. Nevertheless growth of surface mycelium can occur at retail outlets.

Other microbiology

- TVC: plant hygiene, raw materials, competition to starter cultures.
- Water/waste water. Borehole water
- Quality of incoming milk
- Plant hygiene, hand swabs.

Probiotic

- Probiotics: maintainance of bacterial count throughout the product shelf-life.

Applications for Rapid Microbiology

- INCLUDE:
- Detection and semi-quantification of yeasts in less than 24 hours. Moulds in 48 hours
  - Earlier product release
- Monitor effective cleaning and environment
- Monitor levels of probiotic bacteria in product throughout shelf-life.
- Coliform/Entero : <14 hours

Alternative Methods

- alternatives to traditional plating methods.
- Benefits
  - Speed - Faster results
  - Improved repeatability
  - Reduction in errors associated with human error
  - Improved laboratory work flow
  - Reduction in labour and space
  - Increase capacity
  - More independent to skill level of laboratory staff
  - Advancing capabilities
  - Trending data handling

Alternative methods

- “a simple, inexpensive test with a small experimental error, which can be used on a large scale by relatively unskilled workers” Sir Graham Wilson 1935:

The History of Automation in Spoilage and Indicator Testing

- Over last 30 years with 1980's being a particularly active period.

  Automated plate counting

  Metabolic Activity

  Direct Microscopic

  Flow Cytometry

Automated/Assisted Plating

- PetriFoss (Foss Electric) (1970’s)
- Petrifilm (3M)
- Spiral platers

Metabolic Activity

- **Methylene Blue/Rezazurin – Dye Reduction** (1930's; MB adopted by PHLS 1971-for cream)

- **Turbidity:** Using spectrophotometers to perform the measurement from single sample to microtitre formats e.g. Bioscreen Lab Systems. Or those using a centrifugation step e.g. Cobas Bact and Vitek. (1980's)

- **Radiometry** – Bactec 310 and 460 – (Becton Dickinson) C14 released as C14 CO_2 from break down of C14 carbon substrate. (1977).

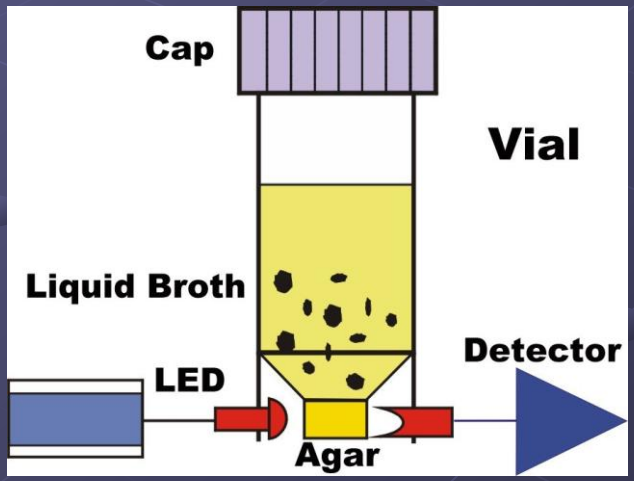
Metabolic Activity

- **Electrical Methods - Conductivity and Impedence** (1980's to present).
  - Malthus™ (Radiometer), Bactomatic (Bactomatic Ltd,) Bactometer ™(bioMerieux), RABIT ™(Don Whitley Scientific) BacTrac™ (Sy-Lab)
- Catabolic activity of organisms break down large molecules into smaller molecules, these metabolic products have a greater charge than the large molecules they originate from. The smaller molecules are more mobile thus conduct more readily.
- Microbial metabolism increases conductance and capacitance of the medium, measured by two electrodes placed in the medium.

Metabolic Activity

- **Rapid Optical Methods**
- BioSys,MicroFoss™
- Soleris™ (Neogen).

- detecting the presence of micro-organisms using optical instruments to detect pH change or CO2 production or other chemical change which alters the colour of a sensitive indicator.



Metabolic Activity

- **Bioluminescence (ATP tests)**
- Celsis, Charm Sciences. BactoFoss (FOSS Electric). (1980's to present)
- Adenylate Kinase (AK) (Celsis)

- The light emitting reaction of the fire fly (luciferin-luciferase) is dependent on presence of adenosine triphosphate (ATP) this reaction is harnessed for the detection of micro-organisms through their ATP content.

- The methods will usually detect at 10^5-10^6 For milk and dairy samples not pre-enriched .
- Care to avoid cross contamination from free ATP

Direct Microscopy

- **The Direct Epifluorescent Filter Technique, DEFT** (Pettifer, 1983) – Staining with acridine orange on a filtration membrane and counted using epifluorescent microscopy,- FOSS Electric

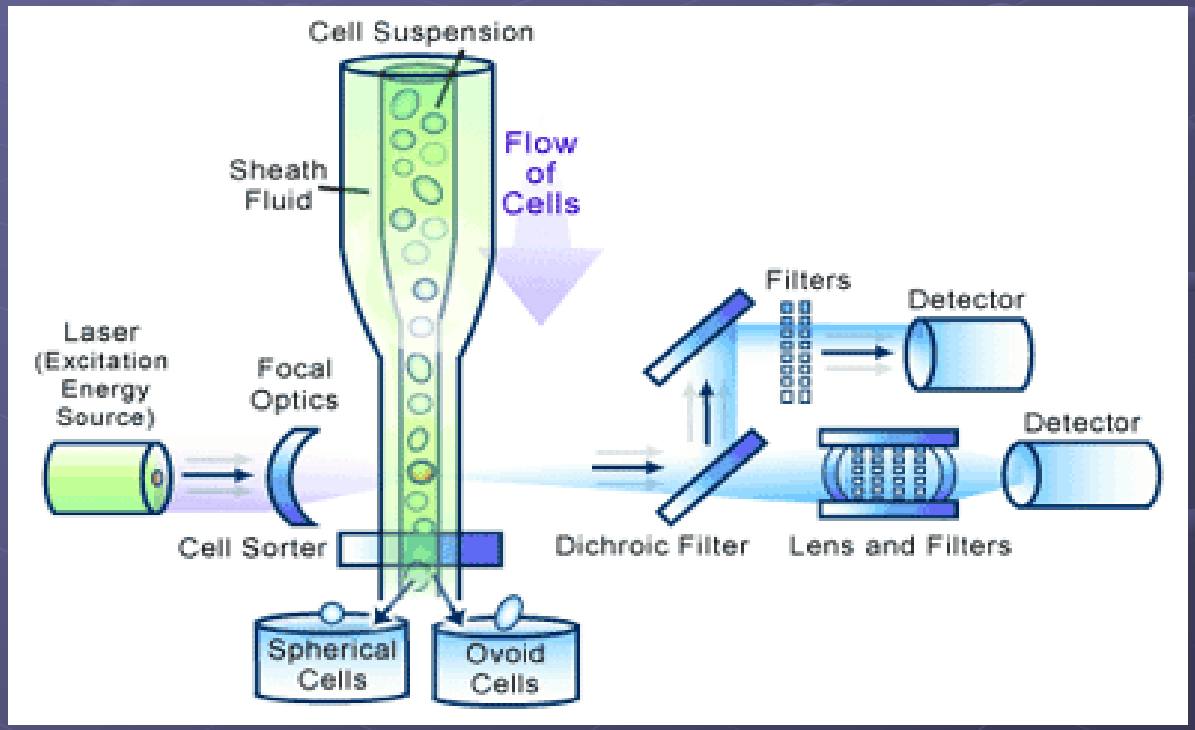
- Bactoscan™ III / Bactoscan™ 8000 FOSS Electric (1980's)

Flow Cytometry

- **Flow Cytometry**
- Automated method for counting stained cells passing in a laminar flow of liquid in the focal plane of a detector. Chemflow/D-Count, Chemunex (AES,now Biomerieux). Bactoscan™ FC (FOSS) (1990's to present).

- Chemflow: Limit of detection claims 100 yeasts/1ml. Pre-incubation of sample required for 24 or 48 hours for detection of 1 yeast in 10g or 100g respectively.

Flow Cytometry



Biosensors

- **Biosensors**

- indicators of biological compounds - which can be as simple as temperature sensitive paint to DNA/RNA molecular techniques. Immunodiagnostics and enzyme biosensors (1980’s to present).

Rapid Automated Systems

- Metabolic Activity:
  - Level for detection: for most metabolic systems around 10^5 cells are required to be present before the instruments can detect.
  - Limit of detection is down to one cell, provided it is viable and capable of multiplying to the detection level of the indicator used in the system.
  - Relationship between time for detection and number of organisms present in original sample.

Challenges to adopting alternative methods

- Comparison to traditional methods
- Demands of accreditation and retailer/purchaser acceptance.
- Cost of implementation and maintenance

Comparison to traditional methods

- In the UK in 1935, Sir Graham Wilson published the results of a three year study ‘The Bacteriological Grading of Milk’.
- Focusing on coliform test and the plate count found them both unsatisfactory. It concluded **‘both methods have a large experimental error and the plate count in particular, in addition to being complex and expensive, gives an appearance of accuracy which is entirely fictitious and misleading’.**

Comparison to traditional methods

- Alternative microbial detection and enumeration systems are compared to the standard plate count (SPC) which lacks precision and was described by Sharpe (1979) as a **“ totally unique datum – nothing in the physical, chemical, biochemical or immunological world corresponds to it and no test based on these properties can ever correlate reliably with it”**

Comparison to traditional methods

- “If it is to be widely accepted and used, a rapid microbiological method should produce results similar to those of existing standard methods. The alternative is to use an indirect test, which may not directly correlate with conventional methods, but does for example with shelf life”. S Pettit (1989).

Comparison to traditional methods

different factors are measured by each method. When comparing a new method with a standard method it should be remembered that the alternative method could be better than the reference method and therefore discrepancies between results do not necessarily mean that the new method is inaccurate. The results of this study have

Adoption of Rapid Methods

- Automated systems need to be integrated into decision making matrix and used intelligently as a tool to assess, monitor and manage microbiological quality control.
- “Building a Picture”
- Requires interpretation.
- PROACTIVITY

Adoption of Rapid Methods

- It takes time to allow a system to settle-in and to ‘build a picture’ using the results obtained. Run at least a year to gather information. Outliers, seasonality
- Information can be acquired and managed directly by localised technicians. Information is available in hours instead of days and weeks.

Adoption of Rapid Methods

- They measure on a ‘different scale’ to plate counts. Can we learn to accept this and establish new criteria?

Neogen Europe

- The Dairy School, Auchincruive, Ayr.
- www.neogeneurope.com
- Soleris System

- 48 Hour Yeast and Mould Test
- Lactic Acid Bacteria Vials