J Ind Microbiol Biotechnol (2003) 30: 239–244
DOI 10.1007/s10295-003-0046-0

ORIGINAL PAPER

Liisa Nohynek · Eija Saski · Auli Haikara
Laura Raaska

Detection of bacterial contamination in starch and resin-based papermaking chemicals using fluorescence techniques

Received: 5 November 2002 / Accepted: 26 February 2003 / Published online: 26 April 2003

Abstract Rapid fluorescence techniques were evaluated for the detection of bacterial contaminants in papermaking chemicals including starch and the resin-based sizes and starch slurries used in the paper industry. Viable and non-viable bacterial cells were visualised by fluorescent probes and detected by epifluorescence microscopy and flow cytometry. The best discrimination ability was obtained with the fluorescent probes LIVE/DEAD and SYBR Green, based on the staining of cellular nucleic acid, and ChemChrome V3, which demonstrated cellular enzymatic activity. The process samples had to be diluted and filtered before fluorescence staining and analysis because they were viscous and contained solid particles. Fluorescence microscopic counts of bacteria in highly contaminated process samples were similar to plate counts, but flow cytometric enumeration of bacterial cells in process samples yielded 2- to 10-fold lower counts compared with plate counts, depending on the consistency of the sample. The detection limits in flow cytometric analysis and in epifluorescence microscopy were 10^3–10^6 cells ml^{-1} and 10^5–10^6 cells ml^{-1}, respectively. Intrinsic bacterial contamination was detectable with fluorescence techniques and highly contaminated process samples could be analysed with fluorescence methods.

Keywords Starch · Fluorescence techniques · Paper industry · Hygiene

Introduction

In the paper and packaging industry, starch and resin-based solutions are used as additives, adhesives, glues and in the sizing and coating of paper [4, 34]. Starch is a white granular substance normally containing two types of polymer structure: amylose and amylopectin. Natural starches are found in most plants in the form of storage granules, which vary in shape and size depending on the plant. An adhesive and highly viscous starch solution is prepared by heating starch powder in water to a temperature exceeding its gel-point, which is from 62 °C to 80 °C, depending on the source of starch [11]. This heating stage, if long enough, usually kills bacterial vegetative cells, but not bacterial spores. Starch is digestible by many fungi and bacteria. Species of the genus *Bacillus* and representatives of enterobacteria are common contaminants in the starch-based solutions used in the paper and packaging industry [22, 23, 26]. The storage tanks or basins containing the starch or resin-based solutions are easily contaminated by biofilm formation on the tank walls, which comes from the materials or chemicals used, or from the elutriating water.

Implementation of the Hazard Analysis Critical Control Point (HACCP) process in the production of food packaging materials shows that the microbiological quality of starch slurries, starch and resin-based glues and other microbiologically sensitive papermaking chemicals used as raw materials in papermaking processes affects the hygiene of food grade paperboard. Therefore, efficient detection of microbial growth in these raw materials is essential [29]. The microbiology of the papermaking process and the raw materials used are usually controlled internally by personnel at the paper mill or by the biocide supplier, with conventional plate-counting methods, often using rapid commercial cultivation methods. ATP bioluminescence and turbidometry, which are indirect methods for the detection of microbial growth or activity, may be applied in a paper mill for internal microbiological control [28]. Obtaining the results of cultivation methods usually takes 2–5 days, which is far too long in comparison with the turnover time in the papermaking process.

L. Nohynek (✉) · A. Haikara · L. Raaska
VTT Biotechnology, P.O. Box 1500, 02044 VTT, Finland
E-mail: liisa.nohynek@vtt.fi
Tel.: +358-9-4565170
Fax: +358-9-4552103

E. Saski
M-real Corporation, P.O. Box 20, 02020 METSA, Finland

Fluorescence techniques can be used for the rapid assessment of viable and non-viable microbial cells in process samples and products for assessing and maintaining the high quality of the products [3, 5, 12, 19]. Fluorescent probes enable the examination of the viability, size and number of microbial cells in various samples. The stained cells are analysed with a flow cytometer or epifluorescence microscope. However, the application of fluorescence techniques in industrial microbiology is still limited. Fluorescence methods have been applied in the food industry for the detection and enumeration of microbial cells in milk or other dairy products [3, 8, 12], in drinks [14, 21], and in fresh and processed meat [9, 20]. Bacteria in natural waters has been analysed using fluorescence techniques [10, 24] and the technique has also been applied in the evaluation of bacterial contaminants in the process waters of the pulp and paper industry [15]. Flow cytometry has also been used to study micro-organisms from biofilms to evaluate microbial population heterogenicity [1].

The aim of this study was to develop fluorescence techniques for the rapid analysis of bacterial contaminants in starch and resin-based papermaking chemicals and to test the applicability of fluorescence methods, compared with the conventional plate-counting method.

Materials and methods

Process sample materials

The test materials were starch- and resin-based sizes, alkylketene dimer (AKD)-size and starch slurries used in coating, obtained from packaging and paper industries producing corrugated board. Samples were aseptically taken from industrial storage tanks or basins and stored at a low temperature (4–10 °C) during transportation and before use.

Microbiological measurements

Aerobic and spore-forming bacterial contamination was enumerated by the plate-counting method from fresh samples, for comparison with the fluorescence methods to be evaluated. The reproductive capacity of contaminating bacteria was determined from samples diluted in peptone saline (Maximal recovery diluent; Lab M, Amersham, UK) and cultivated in duplicate on agar plates, as indicated below. For measurement of the total count of aerobic bacteria, the proper dilutions were plated in duplicate on Plate count agar (Difco, Sparks, Md., USA) or on Nutrient agar (Difco) and incubated for 3–4 days at 25 °C or 30 °C. Enterobacteria were detected on Violet red bile glucose agar (Lab M, Amersham, UK) and spore-forming bacteria were measured on Nutrient agar (Difco) for heat-treated samples (10 min, 80 °C). The agar plates were incubated for 3 days at 30 °C. Some of the samples were fortified to a cell density of 10^5–10^7 colony-forming units (cfu) ml^{-1} with bacteria enriched aerobically overnight in a nutrient broth at 30 °C, using the original process sample as an inoculant.

Pretreatments

Samples were diluted to 1:10, 1:100 or 1:1000 in sterile (filtered at 0.22 μm) MilliQ water or in staining buffer (PBS; pH 7.2). Selected samples were heat-treated (10 min, 85 °C) to control the functionality of the fluorescent probes used to indicate viable and non-viable bacteria.

Fluorescence staining methods

Different fluorescent probes were tested to determine the viable and non-viable contaminating bacteria in the samples. The nucleic acid probes SYTO9 and propidium iodide (PI) in the LIVE/DEAD BacLight bacterial viability kit (L-7012, Molecular Probes, Leiden, The Netherlands) were used as described by the manufacturer. Briefly, 1 ml of sample was prepared in sterile MilliQ water, stained with 5 μl of a 1:1 mixture of SYTO9 and PI fluorescent probes and incubated at room temperature in the dark for 15 min. SYTO9 stains all bacterial cells with green fluorescence and PI, with red fluorescence, penetrates only bacterial cells with damaged membranes.

A nucleic acid probe SYBR Green (Molecular Probes), functioning as SYTO9, was also tested to visualise the viable bacterial cells in selected samples. The probe stains all bacterial cells with green fluorescence (emission at 520–550 nm). The SYBR Green solution was diluted 1:100 in Tris-EDTA (TE) buffer (pH 7.5) and stored in the freezer before use. SYBR green staining solution (10 μl) was mixed with 1 ml of sample diluted in TE buffer (pH 7.5) and incubated in a water bath at 37 °C in the dark for 10 min. To visualise the damaged or dead cells as well, selected samples were first stained with 2 μl of PI for 5 min in the dark and then SYBR Green was added and the was sample incubated as described earlier. The samples were kept on ice in the dark until examined with an epifluorescence microscope or a flow cytometer.

The other fluorochromes tested were ChemChrome V3 (Chemunex, Ivry sur Seine, France) and carboxyfluorescein diacetate (CFDA; Molecular Probes). These are colourless fluorogenic substrates, which are converted into fluorescent products by non-specific esterases in the cytoplasm of the cell. Entire cells containing active esterases fluoresce green, but if the cell membranes are damaged, the fluorescent metabolite leaks out from damaged cells and the green fluorescence is weak or not visible. Briefly, a 1-ml sample was prepared in PBS buffer (pH 7.2), 10 μl of ChemChrome V3 or CFDA esterase substrate was added and the sample was incubated in a water bath at 38 °C in the dark for 10 min. To visualise damaged and dead cells as well, selected samples were first stained with 2 μl of PI for 5 min in the dark and then stained with esterase substrates as described earlier. Finally, samples were kept on ice in the dark for 5–30 min, until examined with an epifluorescence microscope or a flow cytometer.

Epifluorescence microscopy

The staining and viability of the bacterial cells in starch-based slurries and glues were evaluated with an epifluorescence microscope (Olympus BX60; Olympus Optical Co., Tokyo, Japan) provided with a digital image analyser (analySIS ver 3.0; Soft Imaging System, Münster, Germany). The samples were observed using a filter that let through light with green and red-orange wavelengths (filter set 83102x in the 83000 filter set; Chroma Technology Corp., Brattleboro, Vt., USA). The fluorescent cells in each sample were enumerated with a Thoma-chamber cell counter (Knittel Gläser, Braunschweig, Germany), using an epifluorescence microscope. The number of the bacterial cells per millilitre in each sample was counted as a mean value of 5–6 microscopic fields, which each consisted of 16 squares in a Thoma-chamber.

Flow cytometry

The Particle analysing system (PAS; Partec, Münster, Germany) flow cytometer was used for the analysis of process samples supplied with contaminating bacteria stained with fluorescent probes. The PAS flow cytometer is equipped with an argon-ion laser at 488 nm and an HBO-mercury lamp, which enables the detection of particles or cells emitting blue, green, orange and red fluorescence. In addition, particles in samples can be separated according to their particle size (forward scattering) and particle granularity (side

scattering). The stained samples (1 ml) were filtered through 20-µl filters (Partec) before analysis. The running parameters were optimised for each sample by adjusting the gain values of the equipment.

Results

Colony counts

The microbiological contamination of starch- and resin-based samples was quantified with the plate-counting method (Table 1). Resin-based size was the most contaminated by aerobic bacteria (5×10^7 cfu ml^{-1}) of the samples tested. The number of aerobic bacteria in starch-based size was from 5.1×10^3 cfu ml^{-1} to 1.4×10^6 cfu ml^{-1}, depending on the sampling date.

Epifluorescence microscopy

All the fluorescent probes tested visualised bacterial cells in samples, as observed by epifluorescence microscopy. The chemical background in starch- and resin-based sizes and starch solution was quite homogeneous and did not obtrusively adsorb the fluorescent probes used. The stained bacterial cells were easily differentiated from the background, although tested esterase substrates leaked slightly out of the cells and the background fluoresced green if the samples were stored for over 30 min after the staining and incubation steps. The bacterial cells in samples stained with ChemChrome V3 were brighter than those in the CFDA-stained samples; and therefore CFDA was excluded from further studies. Dead or damaged cells were detected with PI staining, which was demonstrated using heat-treated samples.

The detection limit for bacterial cells in epifluorescence microscopy was 10^5–10^6 cells ml^{-1} and therefore epifluorescence microscopy revealed only samples heavily contaminated with bacterial cells (Table 1). For comparison, the number of bacterial cells in the samples was determined by plate-counting. Both the microscopic and the plate-counting methods showed similar numbers of viable bacterial contaminants in starch-based size (1–2×10^6 cfu/cells ml^{-1}) and in resin-based size (1–5×10^7 cfu/cells ml^{-1}; Table 1). In starch slurry and AKD size, the number of bacterial contaminants was below 10^5 cfu ml^{-1} and therefore microscopic fluorescence techniques could not be applied for the counting of contaminants at such a low level.

Flow cytometry

The structure of the sizes and starch solutions tested was viscous and the samples had to be diluted and filtered before flow cytometric analysis. The detection limit for bacterial cells in the flow cytometric method varied from 10^3 cells ml^{-1} to 10^6 cells ml^{-1}, depending on the composition of the sample. As a consequence, bacterial contamination of the original samples was not readily observed with this method. Samples fortified with 10^5–10^7 bacterial cells ml^{-1} were used to test the flow cytometric method for the detection of bacterial cells in sizes and slurries from the paper and packaging industry. Samples were diluted 100- or 1,000-fold before staining to fade out the background and prevent blocking of the syringe and pipes in the flow cytometer.

The original population of contaminating bacteria was detected with flow cytometry in only the starch-based size (Table 1), which was not very viscous. On the basis of colony count and epifluorescence microscopy, there were over 10^6 cfu ml^{-1}. The sample analysed was diluted 10-fold and the viable bacterial population of 10^5 cells ml^{-1} stained with ChemChrome V3 or SYTO9 with PI was differentiated from the background by flow cytometry. The other process samples of resin-based size and starch slurry were prepared with added bacterial cells for flow cytometric analysis (Fig. 1). Bacteria were differentiated from the background of the sample on the basis of the intensity of the fluorescence detected (P1 as green, P3 as red, with fluorescence intensity on a logarithmic scale) and particle size (P5, forward scattering). Fortified cells stained with ChemChrome V3, SYBR Green or PI were readily detected in resin-based size and starch slurry (Fig. 1). The tested sizes and slurries were

Table 1 Bacterial colony counts (cfu ml^{-1}) and viable cell counts (cells ml^{-1}) for starch- and resin-based samples originating from the paper and packaging industry. Samples number 1 and 2 were taken at the same time from separate tanks and sample 3 was taken 6 months later from the same tank as sample 2. For fluorescence microscopy, samples of starch-based size and starch slurry were stained with ChemChrome V3 and samples of alkylketene dimer size and resin-based size were stained with SYBR Green and propidium iodide. For flow cytometry, samples of starch-based size were stained with ChemChrome V3

Sample	Number	Entero-bacteria	Aerobic bacteria		Spore-forming bacteria	Fluorescence microscopy	Flow cytometry
			25 °C	30 °C			
Starch-based size	1	$2{,}5\times10^1$	$8{,}9\times10^5$	$4{,}3\times10^5$	$4{,}0\times10^2$	nd	nd
	2	$6{,}5\times10^1$	$1{,}4\times10^6$	$9{,}0\times10^5$	$3{,}5\times10^2$	2×10^6	10^5
	3	< 10	nd	$5{,}1\times10^3$	$4{,}2\times10^2$	nd	nd
Alkylketene dimer size		nd	$< 10^5$	$< 10^5$	nd	$< 10^5$	nd
Resin-based size		nd	nd	5×10^7	nd	1×10^7	nd
Starch slurry		nd	$< 10^5$	$< 10^5$	nd	$< 10^5$	nd

nd Not detected

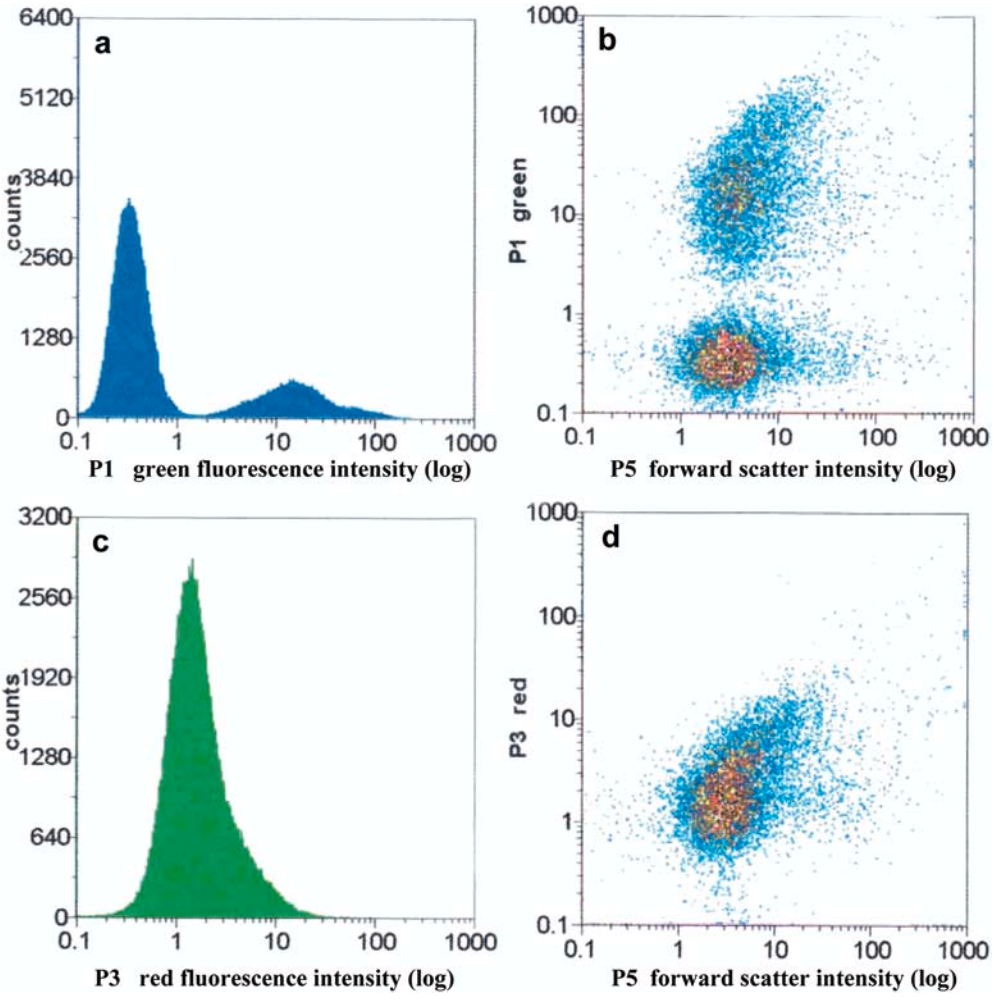

Fig. 1a–d Visualisation of a fortified bacterial population in starch slurry, using a flow cytometer. ChemChromeV3 (stains viable cells green) and propidium iodide (stains damaged cells red) were used as fluorescent probes. **a** Viable cells were differentiated from the background by the high intensity of green fluorescence (P1, fluorescence intensity of 5–200). **b** The sample contained particles and viable bacterial cells of similar size (P5, fluorescence intensity of 1–100) and viable cells were differentiated from particles on the basis of their high intensity of green fluorescence (P1 green, fluorescence intensity of 5–200). **c**, **d** Damaged cells showing high red fluorescence intensity were not detected (P3, fluorescence intensity over 10) and only the background showing low red fluorescence was seen (P3, 0.5–10; P5, 1–10)

homogenous and did not stain strongly with fluorescent probes; and therefore the background detected in the highly diluted samples did not cover the bacterial population in flow cytometric analysis. Enumeration of bacterial cells in process samples with flow cytometry was inaccurate, usually showing 2- to 10-fold fewer cell numbers compared with plate-counting. This may be caused by uneven staining of cells in contaminating bacterial populations in the process samples. In addition, adsorption of the stain by other components of the sample increase the number of events in flow cytometry and may cover the fluorescence of some bacterial cells in the population.

Discussion

Fluorescence techniques have been applied in the paper and packaging industry in the microbial control of process waters [15]. However, application of the online detection of bacterial contamination of process samples with fluorescence techniques has not been reported. In this study, viable and non-viable bacterial cells were visualised in starch- and resin-based glues and slurries originating from the paper and packaging industry, using fluorescent probes based on two different mechanisms, staining of cellular nucleic acid and cytoplasmic enzymatic activity. The fluorescent cells and bacterial populations in process samples were detectable with epifluorescence microscopy and flow cytometry. Bacterial plate counts of process samples were of the same order of magnitude as the viable cell numbers in the fluorescence techniques used, especially in epifluorescence microscopy. However, the dilution steps needed for some viscous sizes and slurries reduced the number of contaminants below the detection limits for flow cytometric and epifluorescence microscopic analysis.

Good correlation between plate-counting and fluorescence techniques used as enumeration methods may arise from the physiological state of the bacterial cells detected from fresh, growing cultures, especially in the case of samples fortified with cells. The physiological state of a bacterial population strongly affects their ability to form colonies on selected agar plates. It has been hypothesised that up to 90% of the environmental bacteria may be non-culturable on the media used, but still exhibit signs of metabolic activity and thus viability [5, 16, 18]. Fluorescence techniques have been extensively studied to elucidate the physiological state of the non-culturable bacterial populations and applied to

reveal non-culturable microbial cells, especially in environmental, medical and food microbiology [6, 7, 19, 24, 25, 30, 31, 32]. Depending on the sampling site, the bacterial populations in some paper and packaging industrial samples probably resemble environmental populations, including cells in different physiological states. In that case, fluorescence methods could give more exact numbers of contaminants than the conventional plate-counting method.

Enumeration of microbial contaminants by epifluorescence microscopy and flow cytometry is a rapid method; and the result is achieved within a few hours, compared with the conventional plate-counting method with its incubation time of one to several days, even weeks. However, microscopic counting may yield consistently higher counts due to the clumping of bacterial cells, because on agar plate these clumps may appear to be only one colony. In studies using milk, Auty et al. [3] reported microscopic counts up to 20-fold higher than plate counts. Enumeration of clumping cells with a flow cytometer is also inaccurate because the samples must be prefiltered and clumping cells and aggregates may be removed before analysis, thereby altering the numbers of cells detected in the sample. However, environmental factors such as pH, ionic profile and water activity may affect the functionality of fluorescence probes and the microscopic counts of viable and dead cells may be lower than the plate counts [3]. In our study, enumeration of bacterial cells by flow cytometry was inaccurate, usually showing 2- to 10-fold fewer cells, compared with plate-counting and epifluorescence microscopy, which may result from components or chemicals in the process samples affecting flow cytometric analysis.

There is a continued demand for rapid, novel methods for the detection and enumeration of microbial cells for industry [13, 17]. Although fluorescence techniques were intensively studied over the past decade and applications have been developed, the number of industrial applications is still low. The facilities for fluorescence techniques are expensive and the personnel responsible for analysis need to be well trained. In addition, staining protocols and parameters for flow cytometric analysis need to be individually adjusted for different samples [19, 33]. Most of the flow cytometric studies reported are performed with the expensive and high-class equipment of research institutes. Compact and even portable flow cytometers have been constructed and a few instruments already on the market may be useful for industrial applications [2, 27]. The factors limiting the use of fluorescence techniques are the high price of facilities and fluorescent probes, the structure of the sample and the sensitivity acquired. Intrinsic bacterial contamination in starch slurries and starch- and resin-based sizes may be detectable with fluorescence techniques, but because of the detection limits of 10^3–10^6 cells ml^{-1} in flow cytometric analysis and 10^5–10^6 cells ml^{-1} in epifluorescence microscopy, only highly contaminated process samples can be analysed with these methods. The fluorescence techniques could be applied in qualification as a rapid pre-screening and sorting method for liquid raw materials in the paper industry.

Acknowledgements Riikka Juvonen is acknowledged for her assistance and knowledge of flow cytometric analysis and Raimo Mattsson for his assistance in microbiological analysis. This study was supported by the National Technology Agency (TEKES), Finland, and the Finnish food and paper industry.

References

1. Álvatez-Barrientos AM, Gaspar I, Blanco Á (1997) Application of cell biology techniques (confocal microscopy and flow cytometry) to the paper industry. In: ICP (ed) Microbiological problems and possible solutions in paper recycling industry. ICP, Ljubljana, pp 70–76
2. Anon (1996) Finding yeast fast. Dairy Ind Int 61:10
3. Auty MAE, Gardiner GE, McBrearty SJ, O′Sullivan EO, Mulvihill DM, Collins JK, Fitzgerald GF, Stanton C, Ross RP (2001) Direct in situ viability assessment of bacteria in probiotic dairy products using viability staining in conjunction with confocal scanning laser microscopy. Appl Environ Microbiol 67:420–425
4. Blumenthal M, Paul CW (1994) Starch-based hot melts for adhesive applications. TAPPI J 77:193–195
5. Breeuwer P, Abee T (2000) Assessment of viability of microorganisms employing fluorescence techniques. Int J Food Microbiol 55:193–200
6. Bunthof CJ, Bloemen K, Breeuwer P, Rombouts FM, Abee T (2001) Flow cytometric assessment of the viability of lactic acid bacteria. Appl Environ Microbiol 67:2326–2335
7. Comas J, Vives-Rego J (1998) Enumeration, viability and heterogeneity in *Staphylococcus aureus* cultures by flow cytometry. J Microbiol Methods 32:45–53
8. Donnelly CW, Baigent GJ (1986) Method for flow cytometric detection of *Listeria monocytogenes* in milk. Appl Environ Microbiol 52:689–695
9. Duffy G, Sheridan JJ (1998) Viability staining in a direct count rapid method for the determination of total viable counts on processed meats. J Microb Methods 31:167–174
10. Gasol JM, Zweifel UL, Peters F, Furman FA, Hagström Å (1999) Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. Appl Environ Microbiol 65:4475–4483
11. Georgeson M (1995) Starch in papermaking. In: Au CO, Thorn I (eds) Applications of wet-end paper chemistry. Blackie, London, pp 76–90
12. Gunasekera TS, Attfield PV, Veal DA (2000) A flow cytometry method for rapid detection and enumeration of total bacteria in milk. Appl Environ Microbiol 66:1228–1232
13. Karwoski M (1996) Automated direct and indirect methods in food microbiology: a literature review. Food Rev Int 12:155–174
14. Laplace-Builhé C, Hahne W, Tirilly Y, Drocourt JL (1993) Application of flow cytometry to rapid microbial analysis in food and drink industries. Biol Cell 78:123–128
15. Lenon G, Craperi D, Deschamps C, Hivert G, Prasse S (2000) Assessment of microbiological activity in water systems by flow cytometry—a new tool using microscopy. Invest Tec Pap 146:605–623
16. Lloyd D, Hayes AJ (1995) Vigour, vitality and viability of microorganisms. FEMS Microbiol Lett 133:1–7
17. Maukonen J, Mättö J, Wirtanen G, Raaska L, Mattila-Sandholm T, Saarela M (2003) Methodologies for the characterisation of microbes in industrial environments. J Ind Microbiol Biotechnol (in press)
18. McDougald D, Rice SA, Weichart D, Kjelleberg S (1998) Nonculturability: adaptation of debilitation? FEMS Microbiol Ecol 25:1–9

19. Nebe–von Caron G, Badley RA (1995) Viability assessment of bacteria in mixed populations using flow cytometry. J Microsc 179:55–66
20. Patchett RA, Back JP, Pinder AC, Kroll RG (1991) Enumeration of bacteria in pure cultures and in foods using a commercial flow cytometer. Food Microbiol 8:119–125
21. Pettipher GL (1991) Preliminary evaluation of flow cytometry for the detection of yeasts in soft drinks. Lett Appl Microbiol 12:109–112
22. Pirttijärvi T (2000) Contaminant aerobic sporeforming bacteria in the manufacturing processes of food packaging board and food. PhD thesis, University of Helsinki, Finland
23. Pirttijärvi T, Wahlström G, Rainey F, Saris P, Salkinoja-Salonen M (2001) Inhibition of bacilli in industrial starches by nisin. J Ind Microbiol Biotechnol 26:107–114
24. Porter J, Diaper J, Edwards C, Pickup R (1995) Direct measurements of natural planktonic bacterial community viability by flow cytometry. Appl Environ Microbiol 61:2783–2786
25. Porter J, Deere D, Pickup R, Edwards C (1996) Fluorescent probes and flow cytometry: new insights into environmental bacteriology. Cytometry 23:91–96
26. Raaska L, Sillanpää J, Sjöberg A-M, Suihko M-L (2002) Potential microbiological hazards in the production of refined paper products for food applications. J Ind Microbiol Biotechnol 28:225–231
27. Roy D, Mulard Y (1996) Rapid microbiology using a laser flow cytometer. Food Technol Eur 3:36–40
28. Salter K (1994) The bioluminescent monitoring of microbial levels in pulp and paper process waters. Pap S Afr, August, p 12
29. Sjöberg A-M, Sillanpää J, Sipiläinen-Malm T, Weber A, Raaska L (2002) An implementation of the HACCP system in the production of food-packaging material. J Ind Microbiol Biotechnol 28:213–218
30. Tholozan JL, Cappelier JM, Tissier JP, Delattre G, Federighi M (1999) Physiological characterization of viable-but-nonculturable *Campylobacter jejuni* cells. Appl Environ Microbiol 65:1110–1116
31. Tsuji T, Kawasaki Y, Takeshima S, Sekiya T, Tanaka S (1995) A new fluorescence staining assay for visualizing living microorganisms in soil. Appl Environ Microbiol 61:3415–3421
32. Williams I, Paul F, Lloyd D, Jepras R, Critchley I, Newman M, Warrack J, Giokarini T, Hayes AJ, Randerson PF, Venables WA (1999) Flow cytometry and other techniques show that *Staphylococcus aureus* undergoes significant physiological changes in the early stages of surface-attached culture. Microbiology 145:1325–1333
33. Winston MK, Davey HM (2000) Flow cytometric analysis of microorganisms. Methods 21:231–240
34. Zijderveld AH, Stoutjesdijk PG (1976) The paper industry. In: Radley JA (ed) Industrial uses of starch and its derivatives. Applied Science, London, pp 199–228