Factors affecting fouling and cleanability of open food contact surfaces

A report on findings from the EU Integrated Project PathogenCombat
Institut Technique Française des Fromages (Actilait)
42, rue de Châteaudun
75 009 Paris
Telephone: +33 1 49 70 72 61
Telefax: +33 4 50 25 82 26

Head manager of Actilait microbiology department
Emmanuel Jamet
e.jamet@actilait.com

Jean- François Chamba
ltff.jfchamba@wanadoo.fr

www.actilait.com

Technical University of Denmark
DTU National Food Institute
Building 227
2800 Lyngby, Denmark
Telephone: + 45 45 25 26 13
Telefax: + 45 45 93 96 00

Lone Gram
gram@aqua.dtu.dk

Alan Friis
alfr@food.dtu.dk

Manchester Metropolitan University
School of Biology, Chemistry and Health Science
Chester St.
Manchester M1 5GD
Telephone: +44 161 247 1206
Telefax: +44 161 247 6325

Joanna Verran
J.Verran@mmu.ac.uk

www.mmu.ac.uk

Agence Française de Sécurité Sanitaire des Aliments
AFSSA, LERQAP
23, avenue du Général de Gaulle BP332
94706 Maisons-Alfort Cedex, France
Telephone: +33 1 49 77 26 46
Telefax: +33 1 43 68 97 62

Brigitte Carpentier
b.carpentier@lerpac.afssa.fr

www.afssa.fr

Roland Cocker
Cocker Consulting
Telephone: +35 3212 3482 12
r.cocker@gmail.com
# Table of contents

Introduction ...................................................................................................................................... 6  
What do we mean when we say...? .................................................................................................... 8  
State of the Art in food-processing organisations ........................................................................... 10  
How does moisture on a surface affect the presence of bacteria, and cleaning and disinfection of the surface? ........................................................................................................... 12  
How does food material on a surface affect the presence of bacteria, and cleaning and disinfection of the surface? ........................................................................................................... 16  
Does the composition of the food material affect the properties of the bacteria? ................... 18  
Does the presence of other microorganisms affect the presence and properties of pathogens? .............................................................................................................................. 19  
What makes certain pathogens persist in a particular factory? ................................................... 21  
Can we make surfaces easier to clean? .......................................................................................... 23  
What are the best methods to use for assessing surface hygiene and cleanability? ............... 26  
Better Training in Hygienic design ................................................................................................. 28  
Improving knowledge of the functional properties of microbes ................................................ 30  
Concluding comments .................................................................................................................. 31
Introduction

PathogenCombat is a large European research project looking at (microbiologically) safe food production. The project is divided into several different parts. One of these parts has focused on hygienic processing systems.

This brochure is about Open Systems - that is where the food processing and handling surfaces are exposed, for example on work surfaces, conveyors, chutes etc, as opposed to pipework (closed systems).

Four groups of researchers in Denmark (DTU Aqua), France (AFSSA, Actilait) and the UK (MMU) have been working together investigating factors affecting fouling and cleanability of surfaces. They used known food pathogens, *Escherichia coli* O157:H7 and *Listeria monocytogenes* for their analysis.

Cocker Consulting Ltd has been working with SMEs on hygienic engineering and design. A second responsibility was the objective of minimising the formation of biofilms and assisting in the development of practical advice on how to remove biofilms once they had formed.

The research groups have been mainly concentrating on issues applying to open equipment, which is used extensively in meat, poultry, fish and dairy processing.
This brochure will provide information and give benefit to SMEs and companies that allow successful intervention and prevention strategies to be designed. We address issues that influence the presence and persistence of food-borne pathogens on open equipment: inherent properties of the strains (persistent versus non-persistent, pathogenic versus non-pathogenic), the influence of food preservation parameters, different surface properties (topography and chemistry), and the presence of resident flora as well as the influence of continuous cycles of cleaning and disinfection.

Obviously many of the basic concepts of food hygiene, hygienic design and hygienic practice in food production are well known and easily accessed (for example European Hygienic Engineering and Design Group (EHEDG) guidelines: www.ehedg.org). Rather than repeating these principles, we have focused our research on particular aspects of concern, so that we can make, where appropriate, additional recommendations to SMEs/companies.

This brochure will outline some of the key findings from the Open Systems group, and translate their significance to the food production industry.

The brochure is divided into sections, relating to the research that we have done. The first section explains the meaning of some of the important terms we have used. The other sections describe our research work and findings. The possible impacts of results with regard to the industry have been summarised and highlighted in blue boxes.
What do we mean when we say...?

**Biofilms** are communities of microorganisms on surfaces, typically encased in some polymer matrix that has been produced by the microorganisms themselves and that can be associated with food residues. Essentially, biofilm may form on any surface exposed to bacteria and some amount of water; they are an important survival mechanism for bacterial cells. Most of the planet’s microorganisms live in biofilms. Attached microorganisms, such as those in biofilms, exhibit different properties to microorganisms that float around in liquid: they are more resistant to antibiotics and biocides; and are of course difficult to totally remove from the surface. That means that the choice of cleaning and disinfection product is highly dependent on the food matrix in which the organism is embedded.

**Interfaces** are where biofilms typically form. The most commonly studied type of biofilm forms at a solid-liquid interface, such as in a pipe, where liquid passes over the surface, bringing nutrient to the biofilm, removing waste, and transferring biofilm cells downstream.

On open surfaces, the interface can also be between a dry surface and the air (solid-air) or between a wet surface and the air (solid-liquid-air), depending on production.
In other words, open surfaces are either always or intermittently wet. If moisture levels are low, cells on the surface are less likely to be growing. However, these attached cells may survive very well, and are able to grow if water becomes available, for example if transferred to a food material.

‘Sub-types’ of microorganisms are members of the same species that differ in some way. For example, *Escherichia coli* is the species name, but 0157:H7 is describing a particular sub-group (serotype) - in this case one that may be dangerous (pathogenic). Other sub-groups of *E. coli* might be non-pathogenic. In other pathogens, such as *Listeria monocytogenes*, sub-types may be equally virulent, but different in their ability to persist during food processing. In our work, we have used different sub-types of pathogens that exhibit different properties.

**Persistence** describes the phenomenon whereby sub-groups of pathogens (or other microorganisms), which may be detected by a DNA fingerprinting method, can be re-isolated repeatedly from the same environment - in other words, they are difficult to get rid of. They can be well adapted to the stress of a given environment, such as a particular factory.

The **resident flora** of a food processing plant describes the typical microorganisms that are normally found after cleaning and disinfection, some of them probably being persistent. Typically these are not pathogenic, but their presence may affect the presence of a pathogenic species. For example, in the meat and dairy industries, we would expect a large resident flora because neither meat nor milk and other dairy products are sterile. We cannot prevent the occurrence of a resident flora, but we can make effort to keep numbers low, particularly in areas where we would expect numbers to potentially be higher (drains, floors etc).

In the stressful environment of a food factory, the microorganisms present have to survive a lot of stressful conditions – transient lack of nutrients, desiccation, adverse temperatures, and repeated exposure to cleaning and disinfectants. Some bacteria can stay alive on these surfaces, but are not easy to grow in the laboratory. These are called **viable but non-culturable microorganisms** (VBNC). Their presence therefore might be overlooked.
State of the Art in food-processing organisations

During extensive visits to food processing organisations across Europe, it was noted that well over 80% of new, CE-marked, food process equipment did not comply with the hygiene provisions of the Machinery Directive, which have been in force since before the year 2000 (the first Directive is dated 1995). Regulators, veterinarians, private auditors and machine suppliers did not have the insight in hygienic design and engineering to assign a probability to the various hazards. They unknowingly accepted an excessive number and magnitude of hygiene risks because the occurrence had also been zero. This was despite the fact that a future occurrence of any one of these hazards could wreck their business and seriously injure or kill consumers.
The following examples were repeated, in large organisations and SMEs alike:

- Regulators and auditors demanding washing of a dry process area.
- An emphasis on performing the ritual of cleaning, rather than on prevention by hygienic design.
- A common target was “visually” clean. This meant cleaning that was not working properly and leaving a biofilm. Some of these biofilms were not visible in normal light, but they were visible using U.V. light.
- Equipment left wet overnight or for a whole weekend after cleaning.
- Footwear poorly cleaned on exiting production areas and on reentering.
- Poor drainage, poor access for inspection and cleaning, wet films, condensation and aerosols.
- There was often a mistaken confidence in the effectiveness of cleaning– most open equipment had many unreachable crevices, yet owners felt that their cleaning was effective.
- Many suppliers, inspectors, veterinarians and auditors lacked hygiene knowledge.
- There were both product safety and occupational hazards, stemming from poor control of moisture.
- The poor moisture control and poor hygienic design was associated with excessive environmental and cleaning costs.
- The user organisations did not understand their rights to have equipment and instructions that could allow them to produce safe food.
- Aggressive fluxes of energy, thermal treatments and chemicals were thought unavoidable. However, hygienic buildings and equipment were needed in order to realise the benefits of “ecological” cleaning methods, longer process times, increased safety and lower costs.
- The nature of customer demand and enforcement often suggested poor knowledge.
How does moisture on a surface affect the presence of bacteria, and cleaning and disinfection of the surface?

**Moisture plays an essential role in the establishment of Biofilms.** Many microbes, including bacteria, can either swim or grow via liquid films. Stagnant liquid conditions provide a ready breeding-ground for microbes and even the use of cleaning sprays may redistribute pathogens from such pools. Unwittingly, manufacturers may assist this by promoting a wet environment. Even the distinctions of product- and non product-contact surfaces, on which the designs may rely for their hygienic performance, can become meaningless under such conditions.

Additionally, wet conditions also result in major injuries as the report of the United Kingdom Health and Safety Executive established 30% of all major injuries were slips and 90% of these slips were caused by wet floors. 95% resulted in broken bones and 1,000,000 days were lost per annum, at an average compensation cost of £4,000 per accident.

There are two forms of **water removal**, (a) the **passive form**, which is considered to be the better way and (b) the **active form** which should be used additionally to the passive form.

(a) **Passive moisture removal** is the result of a preventive strategy, embodied in the basic principles of hygienic design (see the free-to-download Document 8 from www.ehedg.org). The key point is that the equipment and its surroundings need to be almost water-repellent and retain it only on demand, for example by closure of a drain valve or by turning a vessel upright. Big issues are horizontal surfaces, especially downwards-facing surfaces which retain liquids. Many of these downwards-facing surfaces were not easily accessible for inspection or cleaning The Fig. 2 below from the EHEDG Trainers’ Toolbox illustrates this issue and a possible solution.
Surfaces should ideally be accessible for cleaning purposes and inspection. There should not be any unreachable crevices which can be formed by unsealed joints, tack welds and threaded fittings.

(b) **Active moisture removal** usually consumes energy, for example ventilation, extraction, heating and the use of absorbent materials, rubber blades, and, for closed systems, vacuum. It is often necessary after wet cleaning.

*Fig 2: Open Equipment*
For moisture-management, it is recommended to implement a “dry floor” policy.

This means:

- Removing waste at source and rinsing liquid waste straight to drain
- No rubber boots or aprons
- Normal safety shoes
- No boot-washers at production
- Hoses & mops locked during production
- Rubber blades with scoops and bins only, for the removal of waste that falls to the floor.
- Good ventilation
- Controlled wet cleaning where necessary, for example, use of impregnated wet wipes.

A principle difference between microbial contaminants and other contaminants such as chemicals and foreign bodies is that microbes are capable of re-growing after any setback. This is the so called “phoenix” problem. Dilution is perfectly effective for controlling chemical contaminants, for example, but usually offers only temporary relief, where microbes are concerned.

A typical sequence is that crevices and hidden surfaces collect proteins, fats and microbes, so that they could escape effective cleaning and detection, even though 99.99% of the rest of the surfaces are very well cleaned and disinfected. The managers involved conclude on the basis of visual inspection and possible point sampling of accessible surfaces that they have clean equipment.
Each crevice is then a ready locus of contamination, which can lead to dissemination and biofilm formation, and then can turn in more frequent contamination events and finally lead to increased contamination levels. This is especially so if the equipment does not have dry surfaces.

It is worth ensuring that all concerned persons understand the importance and mechanics of biofilm consolidation, which is measured in hours and days and is characterised by physiological and metabolic changes that lead to increased resistance to lethal agents, increased adhesion and an ability to survive in the presence of low nutrient concentrations. There are plenty of examples of such crevices in recently purchased equipment, such as this at an SME dairy showed in the Fig. 3 below.

**Fig 3: Crevices in dairy equipment**
How does food material on a surface affect the presence of bacteria, and cleaning and disinfection of the surface?

On hygienic food contact surfaces, microorganisms from the food, the plant, personnel and environment – some potentially pathogenic – will probably be present alongside food material. MMU (Manchester Metropolitan University) has been investigating the effect of this food material on the removal of microorganisms during cleaning, and has developed methods which allow the food material and the microorganisms to be microscopically visualised and quantified, separately.

Food material should be removed from surfaces during cleaning, with most residual microorganisms present being inactivated through disinfection. The cleaning step is obviously very important: good cleaning will reduce the job required of the disinfectant.

It must be clear that disinfecting is not sterilization. Disinfecting reduces the number of pathogenic microorganisms below a harmful level, while sterilizing eliminates all microorganisms, inclusive spores.

Organic material can interfere with the activity of disinfectants, and can therefore provide protection for microorganisms present on the surface. Food material will always build up on surfaces to some extent, between two cleaning and disinfection routines, and also provide some nutrient to the cells. Growth may be reduced thanks to low temperature, rather than to low nutrient.
During cleaning, both the food material and attached bacteria can be retained in surface features such as scratches, finish lines, joints/welds and pores. Food material particularly can be difficult to remove.

For this reason, we explored ways of detecting both food material and bacterial cells on surfaces. If we were able to separately measure the amount of these two components on a surface, then we can assess how good different cleaning and disinfection procedures are, and also provide a good method for testing novel procedures, new surfaces, and generally screening the hygienic status of plants by examining coupons deliberately placed on site (Fig. 5).

Combinations of stains were used in a new method to successfully differentiate cells from food soil. We used fluorescent stains, and a microscope that allowed examination of fluorescence on a surface. We also measured the microscopic area coverage of a surface by the two components cells and food soil, and were able to assess and compare removal from surfaces.

A new staining method for separately staining food material and bacteria allows plant hygiene, novel surfaces or cleaning and disinfection protocols to be explored. Food material covers test surfaces more widely than bacterial cells, and is difficult to remove. The accumulation of food material on a surface poses problems in cleaning and subsequent disinfection.
Does the composition of the food material affect the properties of the bacteria?

Different food materials will contain a range of nutrients, but also potentially the presence of food preservatives (for example high salt concentrations) will affect the growth or survival of bacteria.

One of the partners, DTU-AQUA, Technical University of Denmark focused on the influence and importance of food preservation factors on the tolerance of *L. monocytogenes* to disinfectants. They also studied whether particular sub-types were more tolerant to disinfectants, and if their ability to cause disease was affected by low concentrations of disinfectants.

Sodium chloride, NaCl, is a common preservative. If *L. monocytogenes* was grown in the presence of 3-5% w/v NaCl, more bacterial cells attached to a stainless steel surface than if they were grown in the absence of salt. Bacteria grown in the presence of NaCl were also significantly less sensitive to disinfectants.

Both of these properties are of importance if attempts are being made to prevent the presence of *L. monocytogenes* in a factory. Measurement of internal cell pH can be used to quantify the effects of a disinfectant at pre-lethal levels. This method was applied during the project, where the internal pH of single cells can be measured. The internal pH is a good indicator of cell viability. Using this method, AQUA were able to measure the protective effect of NaCl, since a slower decrease in internal pH (ie less rapid death) was seen in NaCl-cultured bacteria exposed to Incimaxx (a standard disinfectant) than in bacteria cultured without NaCl.

An awareness of the effect of different components of the food on the health of potential persisting strains, and of their sensitivity to disinfectants is essential. For example, preservatives in the food can increase resistance to disinfectants, and enhance the ability of the cells to stick to surfaces.
Does the presence of other microorganisms affect the presence and properties of pathogens?

The resident flora can affect the presence of pathogens. AFSSA and Actilait were working on the influence of non-pathogenic, resident bacteria on attachment and biofilm formation by *E. coli* O157:H7 (meat) and *L. monocytogenes* (dairy). AFSSA looked at strains isolated from a slaughter hall after cleaning and disinfection, because these were obviously the strains *E. coli* O157:H7 could come in contact with in case of contamination of open surfaces by the pathogen. Using biofilms that comprised of two cultures, *E. coli* O157:H7 and a resident strain, the population of *E. coli* was generally significantly higher than the population of *E. coli* in pure culture biofilms. In other words, *E. coli* O157:H7 was better able to colonise a surface if other microorganisms were present. However, as explained later, a resident strain may be in the majority when repeatedly subjected to the adverse conditions encountered in food factories. Actilait also found that the quantity of *L. monocytogenes* cells that can adhere to PVC or tile was enhanced by the presence of a non-pathogenic biofilm, produced by *Enterococcus faecalis* - although this may be a sub-type specific phenomenon, since AFSSA screened 19 strains of *E. faecalis* and found no enhancing effect on *L. monocytogenes* colonisation.
The non-pathogenic resident flora in a factory can affect the behaviour and the presence of pathogens. Co-culture of resident strains with pathogens may increase or decrease the ability of the pathogen to colonise the surface. It is important to be familiar with the resident flora of a given factory, and with the areas on site where biofilms can form, or cells can become retained on surfaces, so that checks can be made for hygienic status and the presence of pathogens. This is essentially the HACCP Risk assessment process. Reduced moisture will reduce the multiplication and transfer of microorganisms in the environment.
What makes certain pathogens persist in a particular factory?

DTU AQUA looked at the sensitivity of food-borne pathogens to disinfectants, because it has been proposed that persistent bacteria are less sensitive to disinfectants. However, persistent strains of *L. monocytogenes* were not systematically more tolerant to disinfectants than non-persistent strains. Comparing the tolerance of surface attached and planktonic cells to disinfectants, they do not appear to differ markedly in sensitivity.

The partners have collected strains of bacteria from food factories for many years. The strains in these collections allow us to compare their properties, and to find strains that provide a good model for use in experiments.

AFSSA looked at their library of pathogenic and non-pathogenic strains of *E. coli* O157:H7. Two mutant strains (not isolated in food factories) were significantly different from their ‘wild’ counterparts with respect to their biofilm formation in most of the environmental conditions studied.

Populations of non-pathogenic strains were not significantly different in any of the conditions studied, but they were different from the pathogenic strains in some conditions.

Fig 7: A range of different surface materials, such as polyethylene cutting boards and PVC conveyor belts are encountered in a typical food processing environment. The properties of the material can affect their contamination by microorganisms and cleanability.
However, a non pathogenic strain was chosen for use in PathogenCombat, for safe handling, and given to several other partners with the advice that main results are to be validated on a pathogenic strain.

AFSSA focused on the persistence of *E. coli* O157:H7 either in the culturable or viable but non-culturable form with the ultimate aim to assess conditions needed to avoid persistence of a strain because of growth being greater than reduction by cleaning and disinfection. AFSSA investigated the effect of cleaning and disinfection treatments on conveyor belt materials (fig. 7) contaminated once with *E. coli* O157:H7.

When incorrect refrigeration and hygienic practices are applied, sub-population of pathogenic species may survive the stressful conditions on open surface - such as repeated exposure to cleaning and disinfection products. It is thus of prime importance to remove pathogenic cells within the very first days after they have contaminated a surface i.e. before they manage to resume growth and before their resistance to disinfectant increases to such an extent that growth is greater than reduction.

Stress can prevent bacteria from growing on agar plates, and can give misleading results. Cleaning and disinfection can provide stress to cells, but not necessarily kill them. As viable but non culturable cells are in greater numbers than the culturable cells, their detection can be a tool to reveal the presence of a pathogenic bacteria before culturable cells reach a sufficient number to be detected.
Can we make surfaces easier to clean?

MMU has also looked at the properties of the hygienic surfaces, particularly stainless steel, to see how linear features (scratches, or finish) might affect retention of food soil and cells. It is obvious that large defects in surfaces such as gouges, pits, welds, joints or pores will entrap soil and cells. These should be avoided. However, the wear of well cared-for stainless steel surfaces will probably cause increased numbers, orientation and dimension of linear features (scratches). The finish itself will also present more regular, defined features.

Using acetate film, impressions were taken of in-use and new surfaces which enabled the features to be measured, using an atomic force microscope (AFM), which gives very high magnification images without any surface preparation.

The AFM (Atomic force micrograph) also generates data describing surface roughness. We typically use the Ra value, which describes the average departure of the surface profile from an ‘average centre line’. Hygienic surfaces are generally recognised as having a Ra below 0.8 micrometres. However, the use of a statistical measure to describe a surface that may present randomly orientated features of different dimensions might be inappropriate. The AFM images are therefore very helpful, enabling measurement of specific features. We were then able to make surfaces which contained these particular features, and investigate how they affected cleaning and disinfection.
Factors affecting fouling and cleanability of open food contact surfaces

The scale at which our work is conducted may seem to bear little relevance to the food factory where large areas are cleaned, examined for visible cleanliness or hygienic status. However, fundamental investigations of this sort can provide information on the design of new surfaces that might improve cleanability. We showed that the greater the contact area between cells and surfaces, the stronger the retention, in terms of counting numbers of cells attached, but also in terms of the strength of attachment, which we measured using the AFM probe, scanning across the surface with increasing force: the most strongly attached cells required the strongest force to be removed. Soil coverage was higher than cells, and soil was harder to remove from surface features than cells. Food soil also increased retention and growth of bacteria. Cleaning is therefore very important. Surfaces coated with titanium retained less soil and fewer cells than uncoated stainless steel.

It was not possible to identify an end point for cleaning specification over and above the existing Ra 0.8 micron, since smoother surfaces (Ra values below 0.8 microns) appeared to be comparably cleanable.

Fig 9: Cells are retained in surface features
Surfaces should ideally be accessible for cleaning purposes, flat, smooth and hard. Normal wear of hygienic surfaces is unavoidable and cleaning and disinfection choices should be appropriate to remove cells and soil from the surfaces in a given industrial environment. Monitoring of surface wear would be possible with the simple acetate impression technique, newly described. Titanium surfaces might prove more easily cleanable than stainless steel, but the cost implications and the effectiveness in a specific factory environment would require evaluation. It is important to remove food soil from surfaces by ensuring effective cleaning processes.
What are the best methods to use for assessing surface hygiene and cleanability?

The aim of this part of the work was to recommend a specific and simple method for fouling and cleanability assays to industry, particularly SMEs, that is relevant to their product, and whose results are supported by more sophisticated analytical techniques.

The research teams have employed a wide range of laboratory methods to assess factors that influence the presence of bacteria on inert open surfaces and methods to detect them. Our findings have helped us to advise on improved cleaning and disinfection.

We have developed some novel methods for relatively simple laboratory tests, for example the differential staining technique; and the ‘bath’ or ‘swimming pool’ of bacteria incubated on surfaces together with meat exudates.

We have also used some very sophisticated techniques to investigate general principles of hygiene (e.g. AFM, intracellular pH). We also wished to critically evaluate some of the more readily available commercial methods for screening for hygienic status (u/v illumination; ATP bioluminescence). In order to do this, MMU soiled surfaces with complex food soils (meat, dairy), key components of the soils (fats, oils, carbohydrates, proteins), and bacteria, and assess the limit of detection of different commercial and analytical methods.

Fig 10: The ‘swimming pool’ where cells, soil and surfaces are incubated together in order to monitor adhesion and biofilm formation.
U/V detection involves the macro-detection of areas of significant fouling, made readily visible to the naked eye via irradiation using a portable lamp. This method is simple to use, the equipment is portable, and the method is good for inaccessible, soiled surfaces, irrespective of the presence of cells.

The ATP method requires swabbing of the surface (which will probably be virtually visibly clean) for subsequent detection of ATP from cells and soil on the swab via the firefly luciferin-luciferase reaction. This method is well established, simple to use, readily portable, has a good supporting database, and is particularly useful when cells are present, with or without food soil (it is less effective for food soil only due to varying amounts of ATP present). The efficiency of the swabbing method is critical in ensuring that an appropriate sample has been removed from the surface.

Culture of attached cells via removal during swabbing is time consuming, but well-established, although it is susceptible to variation, and is heavily reliant on effective swabbing. If swabbing does not remove representative cells from the surface, a very significant underestimation of contamination will be obtained. An awareness of the presence of stressed and/or viable but non-culturable cells is essential.

Direct examination of surfaces using epifluorescence microscopy enabled differentiation of soil and cells, and assessment of different behaviours in response to surface modification, cleaning and disinfection protocols.
Better Training in Hygienic design

All persons who have direct or indirect contact with the food-producing area (e.g. inspectors, auditors, operators, fitters, Quality Assurance personnel, engineers and designers) need better training in hygienic design. More knowledge is needed in the industry of the hygiene provisions of The Machinery Directive and the derived harmonised standards such as the EN 1672-2 and a range of more specific standards. The standards were written to explain how to meet these provisions.

A scientific basis for designs and for the validation of equipment, such as that of the European Hygienic Engineering and Design Group (EHEDG) is needed. An example for a need of better hygienic design is given in the figure below.

Fig 12: Hygienic design of food processing equipment
It is not easy to recommend a ‘best’ procedure for assessing surface hygiene and cleanability, but general tips may be made for an overall improved knowledge in order to improve hygienic status on open surfaces:

- Inspect for the presence of visible food residues
- Identify critical areas
- Understand key microbiology concepts and terminology and their relevance to your plant (resident bacteria, persistence, preservatives, choice of cleaning and disinfection protocols)
- Select method to detect ‘invisible’ biofilm and food residues on surfaces
- Remember that food soil may be harder to remove than cells, and can affect subsequent hygienic status
- Identify the sensitivity, validity and limitations of a selected method for assessment of hygienic status in specific industry
- When modelling systems for laboratory studies, ensure that the design is appropriate - strain selection and resident strains, growth (biofilm) or survival (immobilised); presence and nature of food soil, impact of repeated soiling and cleaning etc.
Improving knowledge of the functional properties of microbes

It is necessary to understand the properties or behavior of microbes to carry out a hygiene risk assessment, as required by The Machinery Directive. Engineers, designers and others lack knowledge on microbial behavior in relation to hygienic design. Such knowledge is seen as an important background for the hygienic design principles of EN 1672-2.

In the presence of nutrients, the growth-rate of microbes can vary from exponential down to apparent stasis, depending on nutrient sufficiency. The light blue curve in Fig. 13 represent a hygienic design versus an non-hygienic design (dark blue).

Fig 13: Schematic of the growth-rate of microbes in a hygienic (light blue) and a non-hygienic plant (dark blue)
Concluding comments

PathogenCombat has brought together a large and unique combination of expertise across Europe and the globe. New collaborations between researchers have enabled significant progress in the assessment of surface hygiene and cleanability. Our work on the hygienic status of open surfaces continues, using novel surfaces, cleaners, different microorganisms and conditions, and detection of pathogens and more general biofouling in different industries. The potential is enormous.

The findings of our work have been reported in scientific journals and at conferences. This brochure brings the results closer to the end-users, and we hope that they are of interest and relevance to SMEs and companies. They have been validated by intensive experimental work, novel utilisation of state of the art equipment, and movement and exchange of ideas, staff and of course microorganisms. Please do not hesitate to contact us should you require more specific information:

This document has been prepared by Professor Joanna Verran (MMU), with the assistance of Dr. Brigitte Carpentier (AFSSA), Professor Lone Gram (AQUA) and Dr Claire Mariani (Actilait), with many thanks to Drs. Kathryn Whitehead, Nesrine Marouani, Vicki Kastbjerg and Adele Packer.

Manchester Metropolitan University
Dept Biology, Chemistry and Health Science
Chester St.
Manchester M1 5GD
Telephone: +44 161 247 1206
Telefax: +44 161 247 6325
Joanna Verran
J.Verran@mmu.ac.uk
www.mmu.ac.uk
Pathogen Combat with the full title **Control and prevention of emerging and future pathogens at cellular and molecular level throughout the food chain** is an Integrated Project within the EU 6th Framework Programme.

**EU Commission, Directorate E-Biotechnology, agriculture and food**
Scientific Officer Dirk Pottier, SDME 08/85, B-1049 Brussels
dirk.pottier@ec.europa.eu

**University of Copenhagen, Denmark**
Faculty of Life Sciences, Dept. of Food Science
Mogens Jakobsen (Coordinator)
moj@life.ku.dk

Vicki Lei (Project manager)
vil@life.ku.dk

PathogenCombat webpage
www.pathogencombat.com