5 Contamination

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5.1 Introduction

Micro-organisms can be transported through the environment in a number of ways; they can be conveyed in liquids or in aerosols, on particles of solids, or either inside objects or on their surfaces. An object or environment can become contaminated either by direct contact with a carrier of contamination or by contact with some intermediary that has itself has come into direct contact with a source of micro-organisms (Figure 1). Almost anything can qualify as an intermediary according to the definition given above. For example, as Figure 2 shows a person may sneeze into her hand and then transfer viral particles from her hand to a door handle which, as a result, becomes a source of infection. To give another example, a healthcare worker treating a patient infected with antibiotic resistant bacteria may transmit the infection to another patient simply by hand contact. As used here, the term contamination will be taken as referring to the unwanted transfer of infectious biological agents from one location to another. Moreover, the term 'infectious biological agent' is meant to include both prokaryotic and eukaryotic micro-organisms, viruses and prions.

By examining both the routes and the mechanisms by which transfers of infectious agents can occur, it is the intention here to reveal opportunities for optimally applying cold plasma technology either to prevent such transfers occurring, or if that is not possible, to deal with the consequences of such transfers having taken place. In the food industry interventions aimed at preventing the spread of micro-organisms are referred to as 'critical control points', but there is no reason why this concept should

not be applied more generally. Indeed, a number of examples cited below deal with foods. Given the annual toll of death and disease caused by the consumption of microbially contaminated foodstuffs – some 9000 deaths per annum in the United States alone (Mead et al., 1999) - it is entirely appropriate to include such examples within the covers of this book.

A number of workers have examined contamination pathways with reference to certain classes of objects and/or specific types of micro-organism(s). Beuchat (1996) for example, considered in some detail the various ways by which the food poisoning bacterium, *Listeria monocytogenes* could come to contaminate fresh vegetables. As Figure 3 shows there are a large number of, sometimes interconnecting, pathways by which contamination can occur. A few of these centre on animals, but humans, and in particular certain human activities such as the harvesting of produce, can have a role in the transmission of this pathogenic bacterium. The environment often plays an important, but largely passive, role in many potential routes of transmission. As Figure 3 shows the soil can become contaminated with *L. monocytogenes* by a variety of means and can itself become a source of contamination. It is important to appreciate that the sequence of transfer events depicted in the figure need not be instantaneous, and that they can in fact be separated by appreciable intervals of time. The environment has also been implicated as contributing significantly to hospital infections by such bacteria as meticillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant enterococci (Boyce, 2007). The environment in general can then be thought of as a reservoir of micro-organisms. This is to a large extent because, as will be shown below, some micro-organisms have developed strategies for long term survival in the environment.

The objectives here are to examine the processes and mechanisms leading to instances of contamination. This firstly requires some consideration of the associations that micro-organisms can form with objects in the environment and how this affects the ease with which they may be transferred from one location to another. It will also be necessary to gain some understanding of both the physical and physiological states in which micro-organisms can exist and survive in the environment. Sometimes the association that forms between a micro-organism and a particular object in the environment may only be a transient one. An example of this is cited by Todd et al. (2010) where a food preparer changed a baby's diaper and went to work in a central hospital kitchen without changing her clothing. Bacteria from the baby's soiled diaper became transferred to her clothing, these in turn came to be transferred to foods coming into contact with her clothing and this ultimately resulted in some 200 persons suffering from foodborne infections. The association can also take on a more permanent form such as occurs when a surgical wound becomes infected with an antibiotic resistant bacterium. In this instance the bacteria have in effect colonised the wound (McIntosh and Earnshaw, 2009).

The study of the inter-relationships between organisms and their environment is referred to as 'ecology'. This chapter commences with an examination of the microbial ecology of the human environment. There are of course many aspects to such interactions, such as the composition of species present and the interactions that may take place between individual species. However, in this chapter the focus will be only on those aspects that determine how organisms – specifically micro-organisms – come to be transferred from one environment to another.

5.2 The Microbial Ecology of the Human Environment

Definition of just what constitutes the human environment is surely self-evident and barely needs definition – the spaces we inhabit and move through in the course of our own existence and the things we encounter in our everyday lives. As defined, the human environment is therefore not only vast but extremely complex, moreover, there are an almost infinite number of encounters that can be envisaged between humans and their environment and the things within it, almost each one potentially involving some form of microbial transfer. Despite this it is possible to gain some insights into the underlying processes by considering a rather more limited – but still highly relevant – number of specific cases. Therefore, in this section attention will focus on the interactions of micro-organisms with solid objects, and in particular, the *surfaces* of solid objects. It is not intended by this statement to dismiss entirely consideration of fluids, because of course micro-organisms are found in a variety of fluids in the human environment, including those that we are in almost continuous or frequent contact with i.e. air and water. However, for the most part when micro-organisms occur in fluid environments they may be associated with either mineral or organic solid particulate matter (see e.g. den Aantrekker et al., 2003b). Notwithstanding, certain types of cell are to be found in simple – that is unaggregated - suspension in a fluid. For example, the exospores of fungi are distributed through the air as single entities.

The first encounter between a micro-organism and a solid body will, in the majority of cases, occur at the surface of that body. This is of primary significance here because cold gas plasmas can ultimately only inactivate micro-organisms at or near the surface of a solid object. However, certain classes of objects are porous and this may facilitate the transport of micro-organisms into their interiors. To give one

example, there is growing consumer demand for so-called ready-to-eat (RTE) food products - particularly those containing fruit and salad components. These products may become contaminated with micro-organisms during such processes as slicing, dicing etc. Micro-organisms initially present at, say, the surface of a piece of cut fruit, may subsequently migrate via the channels that exist between individual plant cells to the interior of the fruit where they will eventually be beyond the reach of plasma-generated oxidative species (Perni et al., 2008). These workers were also able to calculate the rate of migration and this provided a useful operational parameter - the time after cutting that it would be necessary to apply decontamination technology in order to achieve effective surface disinfection.

The environment in which humans live and function is not of course completely artificial, and it will contain both biotic and abiotic objects – the latter are also sometimes referred to as ‘fomites’. Biotic substances will possess surface characteristics that are intrinsic and dependent on their nature and origins. For example, cantaloupe melons contain surface irregularities, or reticulations, present on their skins which have dimensions measured in the hundreds of microns and are clearly visible to the naked eye. This is significant in the context of contamination because a number of epidemics of food poisoning have been caused by the consumption of cantaloupe melon contaminated with bacteria such as *E. coli* O157 H7, *Salmonella, Listeria* etc. (Bowen et al, 2006). One common cause of microbial contamination was identified above as the soil, particles of which can become trapped in these surface irregularities. However, the characteristics of biological surfaces are not necessarily fixed and may be subject to natural changes. For example, Yang et al. (2005) used atomic force microscopy to characterise the skin of peaches as they underwent ripening. They found that the arithmetic surface

roughness (R_a) of the fruit skin increased with time. Such changes will be reflected in the sizes of particles that can be trapped at the surface. Figure 4 shows *Listeria innocua* bacteria in irregularities present at the surface of chicken flesh.

Abiotic surfaces within the human environment can also undergo changes, as for example when a surface used for butchering the carcasses of animals becomes scored by the implements used for the purpose. Stainless steel is widely used in both food processing facilities and in healthcare environments. Woodling and Moraru (2005) examined the surface topographies of a number of grades of stainless including ones having an electropolished finish, a shot-blasted surface and finally, an aluminium oxide surface layer – this latter having the greatest surface roughness. They estimated that the shot-blasted sample could trap, or 'hide' as they referred to it, a single layer of *Listeria innocua* within its surface imperfections whereas the sample with the oxide layer could potentially accommodate up to three cell layers. However, as will be explained below, micro-organisms are able attach to a perfectly smooth surface, but in the initial stages of attachment relatively low shear forces acting at the surface can result in their detachment. Materials having surface irregularities will display a tendency to trap particles that may themselves harbour micro-organisms and the surface topography may protect the particles and/or micro-organisms from external shear forces allowing them to attach more permanently to the surface.

Gardner and Shama (2000) considered the distribution of spores of *Bacillus subtilis* deposited at the surface of fibrous filter media in relation to UV disinfection of these media. They found that by allocating the spores to a small number (typically 2 or 3) of surface layers, or 'zones', each typified by a particular degree of protection, or shading, from incident UV light, they could model the inactivation kinetics of the spores quite closely. Although as published, the model referred to UV inactivation,

the same concept could be applied to other disinfection treatment including of course cold gas plasma technology. Here the zones could be interpreted more directly as being a measure of penetration into the body of the fibrous media.

Surface roughness, mentioned above, is however only one of the factors determining microbial adhesion, and even this has been contested as constituting a significant factor (Araujo et al., 2010). Also of importance are surface hydrophobicity and surface charge. Microbial adhesion to solid surfaces is conventionally viewed as occurring in two phases. As a micro-organism approaches a solid surface it will begin to come under the influence of van der Waals forces at a distance of about 50 nm from the surface. At closer range still, i.e. between 10 and 20 nm, the effects of additional forces such as electrostatic interactions will start to become significant. At this stage the associations that exist between the micro-organism and the solid surface are entirely reversible - that is, relatively low forces applied at the surface can result in detachment of the organism. At distances of a few nm additional short range forces such as hydrophobic and ionic interactions, and hydrogen bonding take effect and attachment is now considered irreversible. All of these interactions will be influenced by the presence at the surface of the cell of various biological structures such as pili, fimbrae and flagella (Vesper and Bauer, 1986; Fletcher et al., 1993). Abiotic surfaces in human environments are rarely what may be described as being pristine, or perfectly clean, i.e. they may well be contaminated by contact in such a way that residues of various chemical species may be present at the surface. In such cases the surface is referred to as having being ‘conditioned’ and this may not only directly assist in the process of adhesion by for example altering the hydrophobicity of the surface (Midelet and Carpentier, 2002), but if the adsorbed chemical species can also serve as nutrients to the irreversibly-bound organism, the development of a

structured microbial community may develop. This latter term is more commonly referred to as a ‘biofilm’. It will prove useful to consider this process further with reference to Figure 5 which depicts a situation commonly encountered in food processing, that is, the formation of a biofilm within the lumen of a section of process pipeline (den Aantrekker et al., 2003a). After reversible attachment has occurred the now sessile organism may commence the synthesis of a variety of extracellular polymeric substances (EPS) which serve to attach the cells to the surface more strongly still. The composition of these polymeric substances can vary quite widely and will depend on the nature of the micro-organism attaching to the surface and also the predominant physiological conditions at the surface. Notwithstanding such considerations, EPS may comprise polysaccharides, proteins, phospholipids and even nucleic acids. The biofilm itself may take on a variety of different configurations, there are flat biofilms that are able to coat surfaces more or less evenly, and also those presenting a more complex structure. Klausen et al. (2003) have described biofilms formed by the bacterium *Pseudomonas aeruginosa* that have a columnar configuration. Even in flat biofilms micro-channels exist permitting nutrients to diffuse into the film and metabolic end products to diffuse out. Cells within the biofilm are protected to some extent by being surrounded by EPS, and in this state are less susceptible to disinfectants and antibiotics (Holgen and Kolter, 2002). Biofilms can adhere strongly to their substrata and may require considerable shear forces to detach them. As the biofilm depicted in Figure 4 continues to develop it may shed viable organisms into its surroundings and could therefore become a source of contamination.

5.3 Mechanisms of Microbial Transfer

Reference was earlier made in Figure 1 to both direct and indirect means by which micro-organisms can be transferred from a source, or carrier, to an uncontaminated object or environment. Studies such as that of Beuchat (1996), mentioned above, deal by their very nature with potential or putative routes of contamination and not the mechanisms by which transfers of micro-organisms occur. In this section the focus will be on this particular aspect of the contamination process and specifically how micro-organisms may come to be transferred from one location to another. Many of the cases considered here often refer to very specific scenarios. However, this should not detract from their validity and overall usefulness in illustrating mechanisms of microbial transfer, and also from their value in helping to provide insights into transfer mechanisms in cases not considered or anticipated here. Two principal modes of transfer will be considered; the first is the aerial route and the second is microbial transfer by direct contact with contaminated solids or liquids.

Before attempting to do this it will prove useful to enlarge upon a comment made above in relation to how certain micro-organisms have developed strategies for long term survival in the environment. Reference has already been made to the food poisoning bacterium *L. monocytogenes* and the routes by which it can potentially contaminate foods, Gorski et al. (2003) found that *L. monocytogenes* can remain viable in the soil for periods exceeding ten years. Similarly, certain nosocomial bacteria such as *Staphyloccocus aureus* and *Acinetobacter baumanii* appear to be able to maintain viability in an apparent state of suspended animation in healthcare environments for periods measured in months (Kramer et al., 2006). It has even been shown that certain strains of pathogenic bacteria such as *E. coli* and *Listeria*

spp. are able to persist for years in food processing environments apparently surviving the periodically applied decontamination treatments (Holah et al., 2004). Moreover, other micro-organisms have evolved adaptive strategies for survival by producing ‘resting bodies’ – i.e. spores or cysts - that enable the organism to survive long periods of nutrient starvation or unfavourable environments until such time as they undergo transfer to more hospitable environments, or else conditions become conducive to growth. The ability of some micro-organisms to survive for such long periods in the environment serves to highlight the important role which the environment can play in contamination.

It should come as no surprise to find that in the environments specifically under consideration here humans feature quite prominently. With reference to the definitions made earlier, and illustrated in Figure 1, humans (and indeed other members of the animal kingdom) may both be considered as primary sources of contamination and as intermediaries in the transmission of contamination.

Indeed, there are a number of aerial mechanisms of microbial transfer that feature humans. The skin of even healthy people is colonized by a number of different micro-organisms that, taken together, constitute what is generally referred to the normal microflora of the skin. The ‘normal microflora’ of skin is of course not an easily definable term, it will vary, between persons, according to the age of the individual, and between the sexes. Humans shed dead skin cells – or, desquamate - at a rate that is quoted by Beggs et al. (2008) as being of the order of 3×10^8 squamae per day. These workers also estimated that each squamae contained upwards of 100 bacteria. Moreover, it has recently been estimated that approximately 30 % of the population are asymptomatic carriers of *Staphylococcus aureus* (van Belkum et al., 2009). These squamae vary in size, and the median

minimum projected diameter of such cells was estimated by Mackintosh et al. (1978) as being about 20 µm, but the range extended down to below 5 µm. In the case of hospitalised patients a large proportion of these squamae become deposited in their bedclothes. Routine nursing activities such as bed-making can result in the aerosolisation of these squamae. Shiomori et al. (2002) measured the airborne concentration of MRSA in the vicinity of patients infected with this bacterium and found that the airborne concentration increased 25 fold immediately after bed-making. The fact that present on our teeth are biofilms means that every time we speak we generate aerosols that contain micro-organisms – mainly bacteria (Eames et al., 2009).

The organisms that constitute normal skin microflora may be considered as commensal organisms i.e. existing in a relative state of harmony with their human hosts. However, people can of course become infected with pathogenic organisms. For example, if a person is infected with influenza virus, then she will release them into the atmosphere each time she sneezes. The majority of droplets produced by sneezing are about 10 µm in diameter. Rapid evaporation occurs to form droplet nuclei. Such nuclei can be as small as 2 µm in diameter and take over 4 hours to settle a distance of 2 m (Beggs, 2003).

In the course of constructing Monte Carlo simulation methods aimed at predicting the probability of recontamination of a wide variety of food products by the airborne route, den Aantrekker et al. (2003b) collected together much useful data on the levels and compositions of airborne micro-organisms in a wide variety of food processing industries. Unsurprisingly they found higher microbial airborne counts in facilities in which poultry and meat was being processed compared to those dealing with e.g.

dairy or vegetable products. They attributed this to the release of micro-organisms into the air from the feathers and hides of poultry and animals respectively.

Turning to the transfer of micro-organisms from solid objects; biofilms are associated with particularly high incidences of transmission of contamination and it is not difficult to see why. As mentioned above, biofilms consist of micro-organisms embedded within a relatively soft polymeric matrix which normally forms at a solid surface, and relatively low mechanical shear forces are sufficient to detach portions of the biofilm. However, even if the majority of the film is removed and only a small fraction of the biofilm remains at the surface it will retain a capacity for self regeneration provided that the physiological conditions at the surface remain conducive to the continued development of the biofilm. In this way therefore, biofilms can continue to constitute a contamination threat.

Of course transfers of micro-organisms can also occur even when the micro-organisms at a particular surface are not present as a biofilm as some of the examples cited above illustrate. As was also explained earlier, surfaces which are scored or pitted will possess a greater tendency to trap solid particles that may themselves harbour micro-organisms. Once at a surface these particles may become transferred to objects placed in contact with the contaminated surface. The factors that determine the efficiency of transfer are numerous but include the physico-chemical characteristics of the surfaces coming into contact with each other, the characteristics of the micro-organisms whether the surfaces are porous or non-porous, the time of contact, the forces generated during contact and also other environmental factors (Rusin et al.2002; Montville and Schaffner, 2003).

A not uncommon method by which certain types of food can become contaminated is through the use of slicing machines. The sorts of foods typically affected tend to be primarily cold meats (Perez-Rodriguez et al., 2007), but also certain fish products such as ‘gravad’ salmon fillets have also been implicated in instances of food poisoning outbreaks (Aarnisalo et al., 2007). The cross-contamination potential of a number of pathogenic bacteria in transfers both from stainless steel surfaces to foodstuffs and *vice versa*, has been reported in several studies. However, a comparison of the results obtained show how the mechanism of transfer is dependent on the foodstuff concerned, the bacterial pathogens, the original inoculum level and the mechanical forces exerted. However, as mentioned above physical factors such as temperature and moisture levels may also play a role; higher ambient temperatures tend to result in higher transfers of bacteria in the cutting of fatty meats – presumably because fat deposited on the slicing blade becomes more fluid at higher temperatures. Also relevant is the bacteria’s ability to survive on the blade itself – the presence of nutrients and water will prolong the ability of an organism to survive in the environment. It has also been reported that Gram +ve bacteria survive in the environment better than do Gram –ve ones. The consequences of the complex dependencies may be illustrated by comparing ‘transfer coefficients’ i.e. the logarithm of the proportion of cells transferred from the source or ‘donor’ surface to the ‘recipient’ surface. Perez-Rodriguez et al. (2007) obtained values ranging from 0.6 to 2.8 % for *E. coli* O157 H7 and *Staphylococcus aureus* during the slicing of cooked pork, whereas Kusumaningram et al. (2002) obtained values between 25 and 100 % for transfers of *S. aureus*, *Salmonella* Enteritidis and *Campylobacter* from a stainless steel surface to roast chicken and cucumber.

Although any part of the body or clothing can become contaminated through direct contact with infectious agents, much attention has focussed on the hands because they can become contaminated with whatever has been touched or manipulated and this can be transmitted to other persons or objects that are subsequently touched. Mackintosh and Hoffman (1984) constructed what they termed a 'laboratory model' for examining the transfers of a number of bacteria from the hands to fabrics and vice versa. *Staphylococcus saprophyticus* transferred well from contaminated fabric to the hand but not the other way round – the authors quoted a transfer *rate* (*sic*) of 1.67 % which was higher than those obtained for Gram –ve species that included *E. coli* and which varied from 0.3 to 0.5 %. They concluded that *S. saprophyticus* had an affinity for the skin which permitted it to survive well once transferred from fabrics. Similar studies have since been performed with MRSA and the spore-forming bacterium *Clostridium difficile* both of which can cause infections that can prove fatal.

What follows when a transfer of micro-organisms has occurred will obviously depend on the prevailing conditions post transfer. In foods, for example, illness may follow if sufficient organisms constituting an infective dose are ingested along with the food. What constitutes an infective dose is primarily dependent on the type of organism ingested; for example, the infective doses for most serovars of *Salmonella* vary from 10^5 to 10^{10} organisms, but for *Shigella dysenteriae* it can be as low as 10 organisms (Kothary and Babu, 2001). Naturally, disease may still ensue if less than the infective dose becomes transferred to a food if following contamination, the food is stored for a period of time under conditions that allow the micro-organisms that have been transferred to proliferate.

5.4 Determining the Efficacy of Decontamination Processes

It was stated at the beginning of this Chapter that the purpose in examining the mechanisms and routes by which microbial contamination can occur in some detail was to reveal possibilities for interventions aimed at preventing such transfers of micro-organisms. Implicit throughout has been the notion that any such intervention would be based on cold gas plasmas. However, irrespective of the disinfection technology that is used there are some important factors to be considered that have not yet been touched on and which it is appropriate to consider at the close of this Chapter.

The first of these is that in any decontamination process it is necessary to consider how efficacious the prescribed treatment has been. In the majority of cases the technology will have been tested against suitable model organisms, perhaps bacterial spores, and an assessment made of the probability of any organism surviving the applied treatment. Such an approach could not of course guarantee that the target level of disinfection had been met as unforeseen circumstances can often arise, such as for example contamination by particularly robust micro-organisms that resist inactivation. The distribution of organisms on a surface is also a factor; Figure 6 depicts bacterial spores present at a surface in which individual spores are clearly discernable. The application of a decontamination process under such conditions would be more likely to result in efficient inactivation than if the spores existed in clumps and there were diffusive limitations. The advantages of actually being able to assess efficacy directly are self evident; information from such an assay could be used to determine when the treatment had met its objectives. Rapid techniques have in fact been developed for determining whether biological contamination is present at a surface, and such measurements can be related, albeit

in an approximate manner, to the probability of there being living organisms present. One such assay is based on the detection of the energy ‘currency’ molecule adenosine triphosphate (ATP) (Fajardo-Cavazos, 2008). However, what is needed is not only a more relevant assay for viability but a method that could be adapted for real time determination of viability. A particular example would serve to illustrate this point. A brief definition was given at the start of this section of what was meant by the term ‘the human environment’, but the definition given above requires a small qualification here as in reality, it is expanding beyond the terrestrial. The issue concerns the problem of spacecraft decontamination (Debus and Arnould, 2008). The objectives here are first to decontaminate the spacecraft prior to launch to prevent transfer of terrestrial organisms into outer space – a relatively straightforward task. Before re-entry the spacecraft itself and any other objects that may have become contaminated such as sample containers etc. must be decontaminated to prevent the introduction of xenobiotic organisms into the terrestrial environment. In this case however the characteristic of the target organisms (assuming they even exist) will by definition be unknown. They will in all probability be considerably hardier than their terrestrial counterparts coming as they do from environments considerably more extreme than is found on Earth. The inclusion of this particular case here is to highlight the importance of being able to actually determine efficacy rather than to try and predict it. Such technology is under development as part of the current efforts being undertaken for the detection of biowarfare and bioterrorism agents (Lim et al., 2005).

The second important consideration is how exactly does one determine whether a micro-organism is dead – i.e. that a particular decontamination process has met its objectives? Traditional methods are based on assessment of the capacity of the

organism to grow, i.e. form colonies. However, it has long been recognized that failure to grow on synthetic laboratory media is not to be automatically equated with death. Organisms can exist for considerable lengths of time in a so-called viable but not culturable state (VBNC) (Barer and Harwood, 1999). Indeed, organisms in this state can represent a considerable threat. Apart from having evaded conventional methods for detection leading to a state of false security, entry into a state of VBNC is often associated with the retention of various virulence factors (Oliver, 2010). It is in fact not possible to define death in the manner that a coroner might do in human cases. Rather, one may seek to obtain information on particular aspects of cellular physiology such as whether the cellular membrane has become permeabilized or has lost its electrical potential. Even the intracellular pH has been used to furnish information about the condition of micro-organisms (Baatout, 2006).

5.5 Conclusions

The enormous diversity of events and processes leading to the microbial contamination of objects and environments has meant that it has only been possible to consider a handful of specific cases. Notwithstanding, it is hoped that the examples selected for inclusion here give an appreciation of the most important factors underlying all potential transfers of micro-organisms.

It needs also to be stated that whilst the reactive chemical species produced by plasmas are potentially lethal to all living cells, in any novel application under consideration it will prove necessary to definitively demonstrate specific lethality towards the organisms that are likely to be present. It will also be necessary to ensure that the conditions under which this occurs does not result in the compromise

of other critical factors. In the context of foods, this would include ensuring that key nutrients, such as vitamins, were not destroyed.

The application of cold gas plasmas to some of the cases highlighted here would undoubtedly require the development of novel configurations of plasma delivering technologies, but this should not been seen as any impediment to progress as the rate of innovation in this field seems to show no signs of abating – quite the contrary in fact.

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Figures

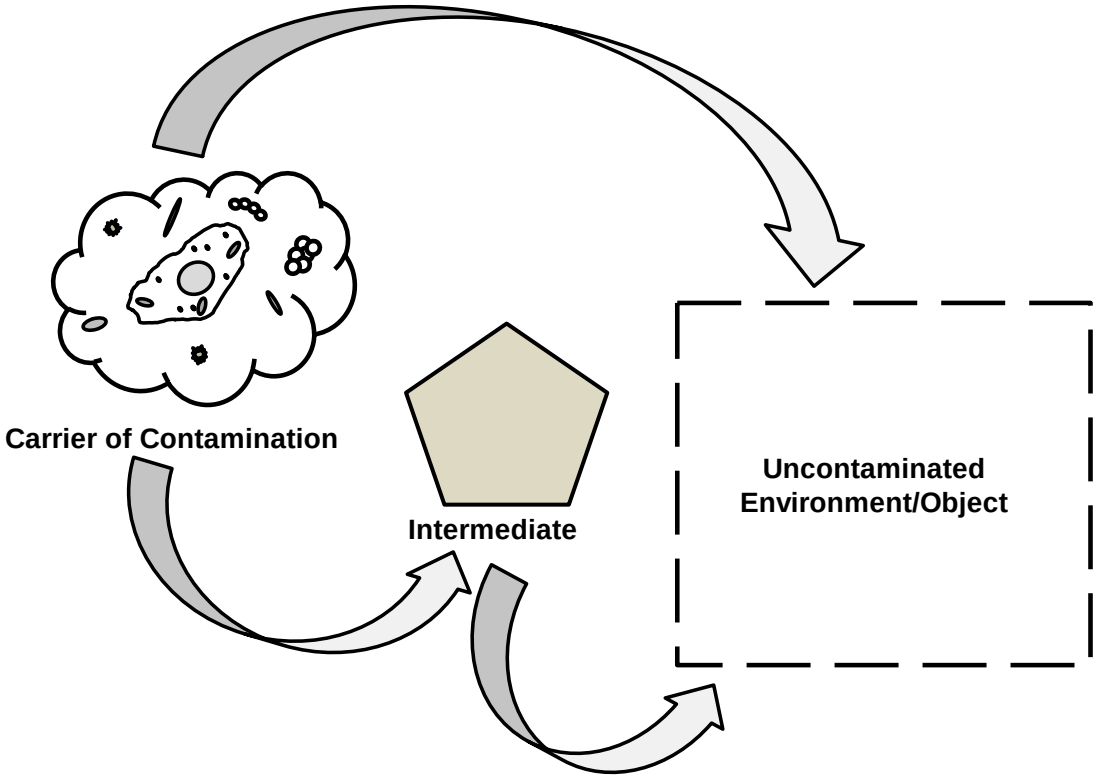

Figure 1 Routes of Contamination

Figure 2 Transmission of Viral Particles to a Door Handle through Sneezing

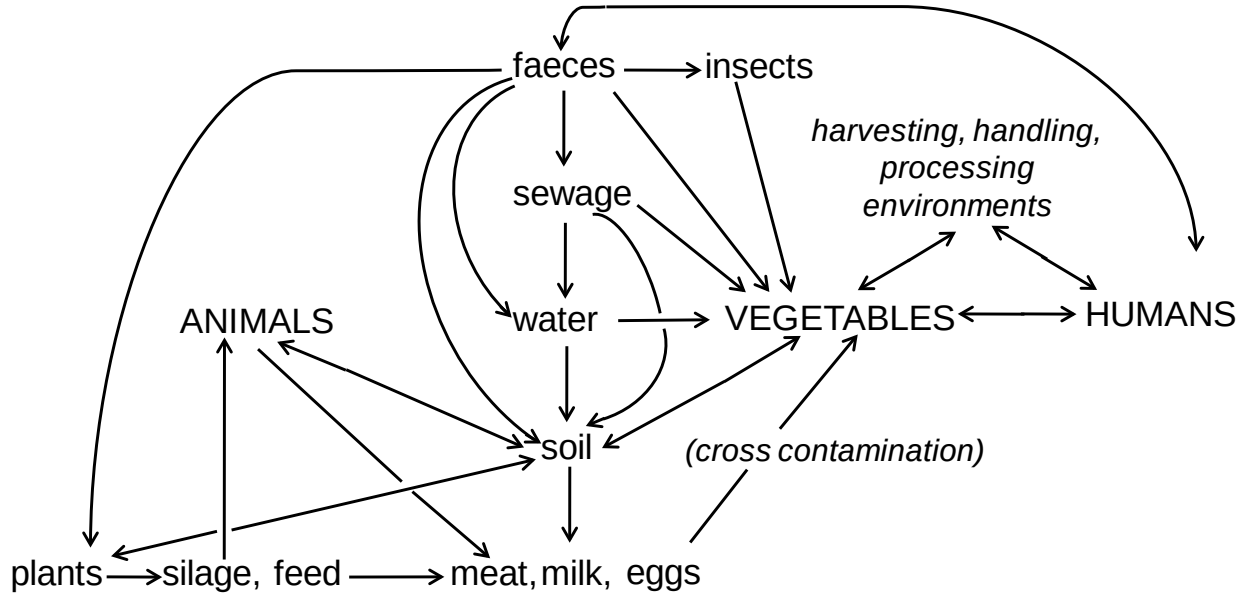

Figure 3 Proposed Routes of Contamination of Vegetables by *Listeria monocytogenes* (After Beuchat, 1996).

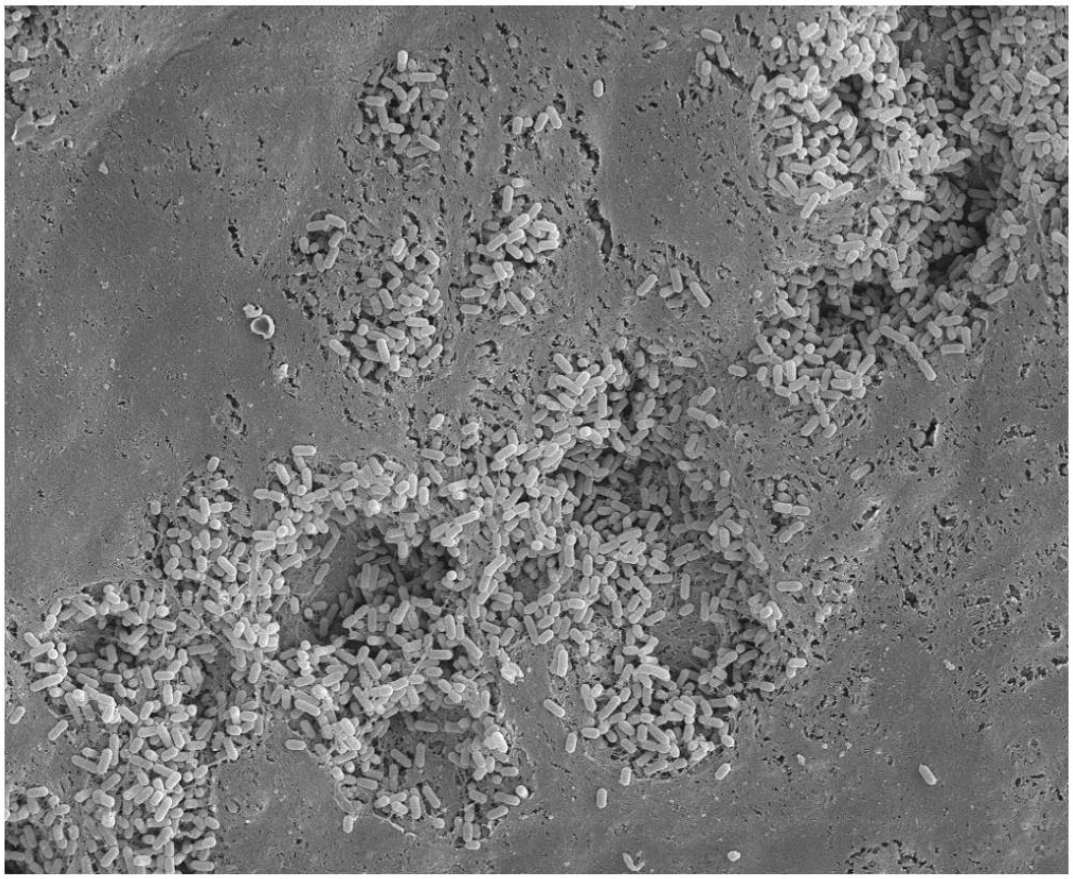
20 µm

Figure 4 *Listeria innocua* on the surface of chicken flesh (Courtesy of Dr Estefanía Noriega-Fernández, University of Oviedo, Spain).

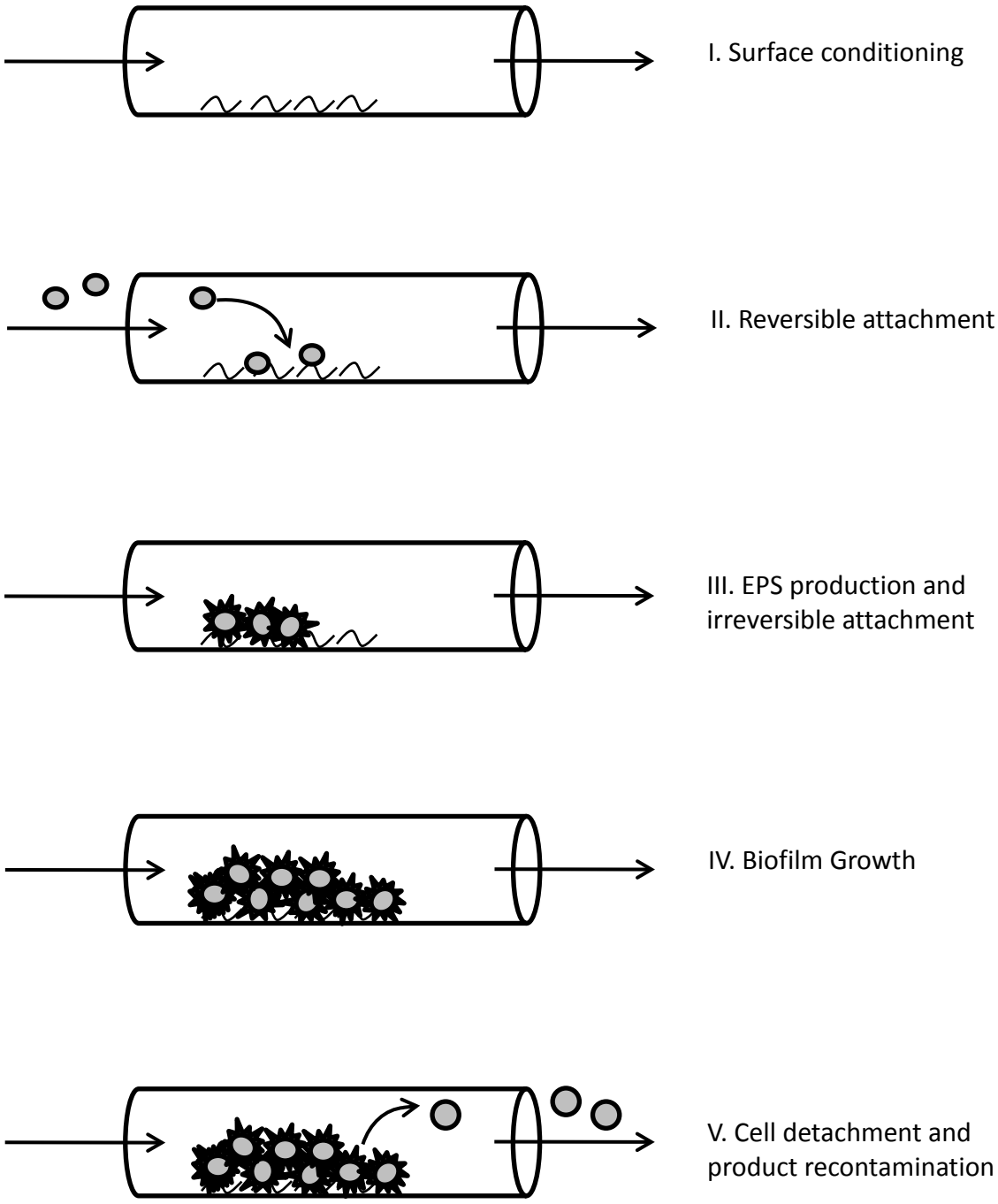

Figure 5 Formation of Biofilm inside the Lumen of a Pipe

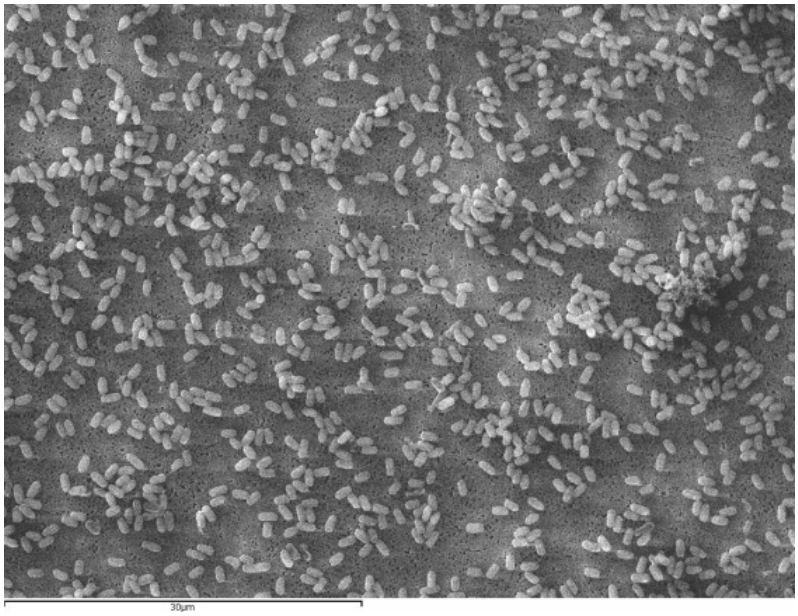

Figure 6 Spores of *Bacillus subtilis* on the surface of a membrane filter (Courtesy of Ms Claire Shaw, Loughborough University, UK.)