4 GAPS IN YOUR ENVIRONMENTAL TESTING PROGRAM

by Stefan Widmann, Product Manager, Romer Labs

1 Viable but non-culturable (VBNC) micro-organisms

What is the viable but non-culturable state?

For a long time, microbiologists assumed that any bacteria that failed to grow on normal culture media were dead. Subsequent research revealed that there is a third state beyond culturable and dead: viable but non-culturable (VBNC). In general, bacteria in the VBNC state do not multiply but are still alive, as shown by their metabolic activity. Most relevant to us is the fact that they can become culturable after resuscitation.

There are many reasons why bacteria can go into the VBNC state; starvation, incubation outside the temperature range optimal for growth, elevated osmotic concentrations, the degree of oxygen concentration, or exposure to white light are just some causes. The specific traits of the bacteria strain in question determine what exactly causes bacteria to enter this state.

Why should food producers care about VBNC bacteria?

Some bacteria able to enter the VBNC state are of concern for food producers. While we do not yet know all bacteria species that can become VBNC, we know some that do; they count indicator organisms (such as Enterobacter aerogenes and Klebsiella pneumoniae), adulterants (such as Lactobacillus plantarum and Lactococcus lactis) and pathogens (such as Salmonella typhimurium and Campylobacter coli) among their numbers.

Having identified them, we must now ask whether these bacteria could return to a fully culturable and potentially pathogenic state. Microbiologists were, for a long time, in the dark on this question, as it is difficult fully to separate VBNC bacteria from culturable ones. Researchers have solved this problem, in part, by using a statistical approach: they dilute high numbers of VBNC bacteria to the point that it is nearly impossible for any culturable bacteria to remain.

The bacteria are then counted after a defined period of time. If high degrees of growth are observed, the only possible conclusion is that bacteria have left the VBNC state and have become culturable. A further corollary is that if they can return to a culturable state, they can also become pathogenic again. There are examples of exactly this phenomena leading to outbreaks. For example, VBNC E. coli O157 were suspected in an outbreak in Japan in 1997, as the numbers of pathogenic E. coli were too low to cause infection.

How can food producers detect VBNC bacteria?

Food producers generally do not have many options at their disposal. There are ways to detect VBNC bacteria, but they are generally not applicable in specific food production circumstances or are too expensive for practical use. Flow cytometry shows some promise, as it directly counts cells and can distinguish between living or VBNC cells and dead cells. Another method would be to apply vital staining in combination with microscopes, but this works only with very clean samples and is extremely time-consuming.

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2 Anaerobic and microaerophilic bacteria

What are anaerobic and microaerophilic bacteria?

Anaerobic bacteria or, more generally, anaerobic micro-organisms, can be divided into three groups: obligate, aero-tolerant and facultative. As their names indicate, they each have special requirements regarding the air, or more precisely, the oxygen, surrounding them. Obligate anaerobes such as Clostridioides difficile are harmed by oxygen and will die shortly after exposure.

Aero-tolerant bacteria such as Clostridium botulinum cannot make use of oxygen and will neither die nor grow in its presence. Facultative anaerobes can use oxygen but do not need it for growth, as is the case with E. coli. There is also the group of microaerophilic bacteria such as campylobacter that need oxygen to grow, albeit in much smaller amounts (1-2%) than in normal air.

Why should food producers care about anaerobic and microaerophilic bacteria?

Several pathogenic bacteria have these special growth requirements. Currently, thermotolerant campylobacter species are cause for worry among public health professionals. On average, every other chicken is infected with campylobacter, making poultry meat one of the most common causes of food poisoning. In the EU, illnesses caused by campylobacter species occur twice as often as those caused by salmonella.

Of the anaerobic group, a Clostridia species such as C. botulinum is responsible for the foodborne illness known as botulism, often transmitted through canned (i.e. oxygen-poor) food, in which C. botulinum can thrive and produce the compound botulinum, which is toxic to humans. Another Clostridia species, C. perfringens, is the most common source of food poisoning in the US and Canada and causes symptoms such as abdominal cramping and diarrhoea.

The risk of C. perfringens infection correlates especially strongly with food kept or stored in warm conditions for longer periods of time, which favours their growing to infectious numbers (10^4 cfu/g).

How can food producers detect anaerobic and microaerophilic bacteria?

While it is possible to detect all groups of anaerobic and microaerophilic bacteria with classic agar plates, they, with the notable exception of facultative anaerobes, can grow only under carefully controlled oxygen levels. For this reason, a common aerobic plate count (APC) will not detect these organisms.

An additional concern is time: as with any other agar plating method, the incubation of anaerobic and microaerophilic bacteria takes at least two days before presumptive results are reached. By this time, a contaminated product may have already made its way to consumers.

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3 The Great Plate Count Anomaly

What is the great plate count anomaly?

Only 1% of bacteria can be cultivated with the knowledge and techniques currently at our disposal. The 'great plate count anomaly' is the term we use to describe the observation that microscopic cell counts are significantly higher than corresponding counts of 'colony forming units' on agar plates.

A couple of examples can illustrate this phenomenon best: while 50% of the micro-organisms of the oral flora can be cultured with agar plates, most of the gastrointestinal flora cannot be cultured at all. The reasons for this are numerous, but the organism community surrounding the bacterium in question, including other bacteria as well as plants and animals, may play an important role.

Aerobic plate count methods rely on very general supplements, which exclude most bacteria groups. Technically, this is not really part of the great plate count anomaly, as some bacteria are able to grow on special agar plates under special conditions (such as anaerobic or microaerophilic conditions).

Why should food producers care about the great plate count anomaly?

The great plate count anomaly does not pose significant problems in day-to-day testing runs, as aerobic plate counts are specific to a given production environment and, as such, are always relative to an established baseline determined for that production environment.

However, plate methods are very time-consuming, requiring an incubation period of up to three days, depending on the protocol in effect. There are direct methods that do not require a cultivation step to count bacteria: microscopes provide a comprehensive view of bacteria but are also very time-consuming, while flow cytometers currently on the market are expensive and require intense training to master.

While such direct methods are common in water treatment facilities, they are not very common in the food industry. There, instant counting technologies must be correlated with established agar-based methods, which are still the accepted standard for environmental hygiene management.

How can food producers detect what agar plates can't?

Thanks to advances in technology, formerly bulky and unwieldy devices are becoming portable, more user-friendly and more affordable. This is also true of direct counting methods that can detect all intact bacteria in a sample.

Broadening the scope of bacteria detected and, crucially, reducing or eliminating time otherwise spent waiting for incubation can help shift the focus of hygiene monitoring from post-production to pre-production.

In this way, food producers can reduce the waste associated with products with a shorter shelf-life and of greater concern regarding hygiene, such as fresh meat and dairy products.

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4 Psychrotrophic bacteria

What are psychrotrophic bacteria?

Psychrotrophic bacteria have the ability to grow at temperatures as low as 4°C with optimal and maximal growth temperatures above 15°C. This characteristic makes such microbes especially problematic for foodstuffs and beverages such as raw meat and milk stored at low temperatures for longer periods of time.

The psychrotrophic groups of bacteria most commonly found in food are the genera Pseudomonas, Aeromonas, Achromobacter, Serratia, Alcaligenes, Chromobacterium and Flavobacterium spp., as well as Gram-positive genera such as Bacillus, Clostridium, Corynebacterium, Streptococcus, Lactobacillus and Microbacteria.

Why should food producers care about psychrotrophic bacteria?

Psychrotrophic bacteria are adulterants and can significantly diminish the quality and, in particular, the shelf life of food. Chilled production facilities and storage tanks offer the perfect environment for the multiplication of these bacteria species. In chilled milk, for example, Pseudomonas fluorescens can produce both proteases and lipases.

Hence, Pseudomonas is regarded as a species typically responsible for technological troubles, as the proteases and lipases they produce can cause milk fat to degrade, giving milk a greyish colour and bitter taste. In vacuum-packed, refrigerated raw meat, the microflora is dominated in most cases by psychrotrophic lactic acid bacteria.

Psychrotrophic Pseudomonas species are the micro-organisms most often responsible for spoilage in aerobically stored chilled meat. It is well known that Pseudomonas spices are very robust and able to withstand stressful environmental conditions that would inhibit the growth of other spoilage micro-organisms.

How can food producers detect psychrotrophic bacteria?

ISO standards for the enumeration of psychrotrophic bacteria (ISO 17410:2019) require an incubation of 10 days at 6.5°C. This is not a practical timeline for analysing a product before it is sent to the vendor or customer. There is also a standard method (defined in ISO 8552) that merely estimates the quantity of psychrotrophic bacteria that takes 25 hours to get results.

However, this is not a rapid method, and is not suitable for cleaning verification as it only provides an estimate of contamination. Flow cytometers include psychrotrophic bacteria in their counts and can give results within minutes.

However, most cytometers are expensive and bulky and are used mainly in the milk industry to test large batches of milk simultaneously. In their current state, they are not applicable to in-process hygiene management or other industries such as meat production.

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6 GAPS IN YOUR ENVIRONMENTAL TESTING PROGRAM

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5 Identification and detection of foreign bodies

What are foreign bodies?

Foreign bodies are among the chief sources of customer complaints concerning food products. According to the EU's RASFF (Rapid Alert System for Food and Feed), the presence of foreign bodies is the third most frequent source of complaint, preceded only by that of pathogenic micro-organisms and allergens. Even in the best-managed production facilities, there is always the risk that fragments from production equipment or packaging materials may inadvertently end up in the final product. The impact on food quality can be more than just an aesthetic concern; foreign bodies can cause life-threating incidents. The three most frequently reported kinds of foreign bodies are plastic, metal and glass. These hard materials can cause injury to the human digestive tract and, in some cases, can be toxic. Foreign bodies are typically found in ground or bulk raw materials such as cereals or flours as well as in processed foods. Glass fragments, for example, are usually found in products packaged in glass, where accidental damage can lead to contamination by foreign bodies.

Why should food producers care about foreign bodies?

Foreign material of any kind can potentially introduce a physical hazard into a food product. European legislation requires that food producers comply with various hygiene regulations to ensure that food is safe and hazard-free. This includes the reduction and elimination of foreign bodies in food. In the USA, hard or sharp foreign objects are legislated as adulterants; the failure to prevent them from entering the final product can result in prosecution by regulatory authorities. The resulting damage to the brand of the producer can be considerable.

How can food producers detect foreign bodies?

Visual inspection, while important as an initial step, can be subjective; its effectiveness is subject to human error and can be influenced by any number of factors, such as lighting, heating, ventilation and noise. Highly sensitive, automated systems are generally the industry standard. Some frequently used technologies on the market that can detect certain foreign bodies include thermal imaging systems, metal detectors and X-ray systems. These systems are well suited for various larger objects that may enter the food production process. Metal detectors, of course, are not able to detect glass or plastics. Both X-ray and IR-imaging can distinguish larger contaminants from the food product thanks to the their differing energy spectrums.

These technologies, however, cannot detect the smaller particles caused, for example, by abrasion from parts in production machinery. Furthermore, the efficacy of these technologies can be vouchsafed only if most of the final food product is able to pass through the limited detection area of the systems. The market currently lacks a solution that can detect microscopic materials during the production process and as part of cleaning verification. Small microscopic particles, whether introduced by abrasion or by the use of cleaning equipment or cleaning agents, could represent a serious risk to consumers in that they may indicate the presence of larger foreign bodies in the production line. As part of a general cleaning programme, the monitoring of microscopic particles can minimise the contamination risk from larger foreign bodies.

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6 Biofilms

What are biofilms?

Micro-organisms are able to colonise surfaces by forming a polymeric matrix in which multiple microbial species may be present; this is known as a biofilm. Evidence shows that the ability to form and survive in biofilms is not restricted to specific groups of micro-organisms. In fact, the vast majority of bacteria are able to form biofilms. Biofilms may therefore be composed either of monocultures or of several different micro-organism species.

Some researchers have suggested that the complex structure of mixed biofilms renders them more stable and more resistant to cleaning chemicals. The initial population that binds to the surface can change the properties of that surface, allowing for bacteria that come later to adhere via cell-to-cell association; in some cases, the attachment of a second species may increase the stability of the biofilm population. For example, studies show that L. monocytogenes is more likely to adhere to steel in the presence of Pseudomonas.

Why should food producers care about biofilms?

Biofilms that form on food processing equipment and other food-contact surfaces act as a persistent source of contamination, threatening the overall quality and safety of food products and possibly resulting in foodborne diseases as well as economic losses. Spoilage micro-organisms are known to be responsible for almost a third of losses in the food chain supply, making biofilm prevention and control a priority in the food industry.

Micro-organisms that form or thrive in biofilms are more resistant to disinfection, making them problematic in a wide range of food industries. Other effects of biofilms such as the corrosion of metal surfaces are a further critical concern in the food industry. In either case, the presence of biofilms in a food production facility puts human health at risk. The degree of risk is dependent on the bacterial species forming this three-dimensional, living structure.

How can food producers detect biofilms?

The standard methods for detecting biofilms are still agar-media based. Surfaces can be sampled directly with dip-slides and contact plates or indirectly when using swab sticks, sponges or swab cloths. Indirect methods require that dilutions be prepared and spread out on petri dishes. In both direct and indirect methods, it takes days to get results.

ATP test systems can provide faster results, but kinetic data from freely suspended planktonic cells should not be used as a reference for biofilm detection as the release of ATP is much lower for biofilms. Accordingly, ATP devices used to detect biofilms tend to have a much higher limit of detection, meaning that they are not as sensitive as they would be when detecting free floating bacteria.

At the moment, there is no instantly secure way to detect biofilms before a production run begins. Direct cell counting technologies can help to close the gap as they can detect bacteria-forming biofilms instantly without the compromises that must be made to accommodate ATP systems.

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