

Contamination Concerns

Marcin Los at Phage Consultants examines the causes of bioprocess contamination and outlines steps that can be taken to avoid it

Contamination in bioprocesses is a risk that is frequently underestimated or even overlooked when people calculate the costs of running a business based on the use of microbial or eukaryotic cells. One of the main reasons for this is a lack of information, caused by at least two independent factors. Firstly, contamination (from a purely scientific point of view) is considered a failure, and thus it is hard to publish the results of experiments in which contamination problems occurred – in fact, they are not usually considered worthy of publication. Even case studies of contaminations in bioprocesses are seldom reported in scientific journals. Secondly, from a business point of view, a confession that a company suffers from any contamination problem is often regarded as a form of suicide, as it is easy for competitors to use this information to their own benefit. These two aspects contribute to the fact that the problem is not taken seriously enough by both decision makers and production staff.

CONTAMINATION TYPES AND THE WORST SCENARIO

Each type of fermentation shows a different susceptibility to contamination. Fungi fermentations – yeasts for example – are mostly endangered by bacteria; animal-derived cell cultures suffer from viral, bacterial and, to a lesser extent, fungal contaminations. When bacteria are used for production, the most dangerous contaminants are bacteriophages, but other bacteria, especially sporulating ones, also pose a threat. Although some contaminations seem easy to avoid, practice shows that this is not always the case. It is very important to be prepared for this problem and to have a ready-to-use procedure, which should be implemented as soon as contamination is detected. A frequent contamination scenario is that the facility runs for a long time (even several years) without any problem, and then equipment failure or operator error causes an initial contamination. A single fermentation is lost, but due to a lack of proper treatment, contamination spreads within the facility. This in turn usually means that all weak points, masked so far by lack of contaminant in the facility environment, will be an entry point for contamination to recur. In the worst scenario, the facility will become unable to continue production, as every fermentation is contaminated and will need to be destroyed, increasing the contamination level. Breaking this loop may be difficult when the personnel have little or no experience in dealing with such a situation.

PRIMARY CONTAMINATION

The source of primary infection is usually not easy to detect, and is often impossible to identify unless the contamination originated from serious equipment failure. This is due to the fact that the spread of the contaminant after infection may mask the original source of the problem. However, one clear conclusion can be made on the basis of contamination occurrence – there are weak points to be fixed. In the case of some contaminations (sporulating bacteria and bacteriophages) there is a seasonal dependence in the frequency of occurrence, with peaks during spring and autumn (1,2). This

pattern can be linked with agricultural works, which may greatly increase the amount of contaminants released from the ground and spread widely. Relatively large numbers of contaminants circulating in the environment means that it is much more likely that they will pass through the barriers, protecting the bioprocess. Of course, the better the protection, the lower the probability that contamination will occur. However, at some point, it is usually necessary to balance the costs of protection and the increase in security.

SECONDARY CONTAMINATION

Secondary infections originate from the spread of the causative agent from the area of primary infection in the facility. The entry paths of secondary contamination to the process are usually much harder to eliminate, as a high load and frequent occurrence of the contaminant may increase the chances of penetrating the barriers, which otherwise may have successfully stopped infection. The much higher numbers of contaminant also mean that it is more difficult to eliminate: 1ml of bacterial culture may harbour as much as 10^{12} - 10^{13} bacteriophage particles (3). Spillage of that amount of contaminated material may occur relatively easily and may go unnoticed, but it means that the likelihood of another process becoming infected is raised dramatically. A full-scale outbreak may also be triggered, with the result that the ability of the facility to run even a single successful fermentation without contamination is reduced nearly to zero. Even worse, contamination may be transferred along with any material transfer, for example a cell bank, to another facility.

EVOLUTION OF THE CONTAMINANT

To make the situation more complicated, even evolution works against the contaminated facility: the contaminant is constantly evolving to use the available resources, in the best possible way. The majority of fungi, bacteria and viruses, especially bacteriophages, are not well adapted to an environment with a high abundance of resources (nutrients, host cells), due to the fact that such conditions do not occur in nature (4). During the initial contamination, natural selection favours the mutations of the contaminant which make the best use of this extremely rich production environment. These mutants will give more progeny and increase the speed of contamination. Usually, this is observed as a reduction in generation time, and in the case of viruses, an increase in progeny formation ratio and the resulting burst size. After contamination, those which can survive better in a hostile environment, subjected to drying and various cleaning procedures designed to kill the contaminants, tend to be sporulating bacteria and drying-resistant viruses. The most common bacteriophages causing outbreaks in fermentation facilities are T1-like phages, which usually have a very high resistance to drying.

HOW TO PREVENT CONTAMINATION

Contamination can happen in any type of facility. Even biopharmaceutical facilities that are run under a cGMP and

HACCP regime are still prone to occurrences. However, there are several ways of decreasing the probability of contamination which should be used concurrently. The factors to consider are facility location, facility construction, process design, choice of equipment, work organisation and personnel skills. All these elements should be considered capable of increasing or reducing the probability of infection and a subsequent outbreak.

The influence of facility location on the risk of contamination is quite obvious, as the greatest source of contaminants is outside the factory. Thus, for example, it is not recommended to locate *E coli* fermentation plants near water treatment plants and animal farms. The general rule is not to place the facility operating the process based on particular organisms near to a place where the same organism exists in great abundance in natural reservoirs, as it usually means that its natural enemies are also present.

One of the most important issues is the design of the facility. There may be some avoidable mistakes that have occurred as a result of the architect's ignorance of how to reduce the spread of the contamination and the contamination sources. There should be close co-operation between the architects and contamination experts during preparation of the plans. If this is not accomplished, the risk of primary infection may be increased considerably; facilities may not be prepared for effective cleanup, or may even simplify the spread of the contaminants. As a result, the costs of operation of such a facility may rise dramatically due to contamination problems, and the expected profit may not be

generated due to decreased productivity. The situation may be improved to some extent by rearrangements to the existing facility, changes in work habits and organisation, and changes in process design and the equipment used.

When designing the process, it is always good to minimise the risk of infection and increase the chance of early infection detection. In some cases, these two aims are contradictory; for example performing a multi-step fermentation with a stepwise scale-up increases the chance of early detection of contamination before the final scale-up, at the same time as increasing the chance of contaminating the process due to multiple material transfer steps.

Sometimes even a single piece of equipment may determine the safety of the whole process. In order to choose equipment properly, it is necessary to answer the question: what type of contaminant may endanger the process? In general, viruses are the most problematic contaminant as their small size and very high loads after multiplication in the host cells mean that they are very difficult to remove. However, their susceptibility to various treatments is different to bacterial cells and spores. Thus to effectively protect the process, all types of possible contaminations should be taken into consideration and proper methods of prevention should be used.

Being well organised can greatly reduce the risk of contamination and time necessary to recover. It should fit well to the specific conditions of a facility and to the process demands. This includes

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proper design of standard operating procedures and work practices (1,5). It is important to have emergency procedures, which are easy to implement and lead to the reduction of the spread of the contamination, but these are frequently omitted. Their use should be conditional, and conditions of use should be clearly defined.

Even in the best designed facility, with the most advanced equipment that is perfectly fitted to the process, there is always a risk that the personnel will be a weak point that will lead to an infection. Although not generally the case in the majority of facilities, it is still possible that people running the process have a very limited knowledge of contaminants, the way they may be introduced to the process and the way they can be recognised and eliminated. This makes it less likely and much more complicated to avoid contamination; even perfect procedures may fail if operators do not understand the meaning of key steps. The lack of understanding also greatly reduces the probability of rapid recovery after contamination, as it can be spread involuntarily by personnel or not efficiently cleaned. It is very important to understand that the personnel are the first and the last line of the defence against contamination, and proper preparation and education as to how to fulfil this function is absolutely necessary.

DEALING WITH CONTAMINATION

Sometimes contamination is not easy to recognise – it may manifest itself with a change in oxygen consumption, optical density growth, pH, product formation or foaming when compared to uncontaminated processes. Even a viral contamination of the process may not always give obvious signs, so collecting samples from the processes and performing proper tests is highly recommended. The most recommended option is testing samples from all process runs for the presence of contaminants. Tests should be performed by properly trained personnel, especially when checking for viruses, as obtaining a false negative result in these types of tests is relatively easy, when conditions are not properly prepared.

If contamination has already occurred, the main task is to prevent its spread in the facility. Collection of a sample of contaminated material from the fermentations which failed is crucial, but often omitted. This action requires proper precautionary measures to be undertaken, but is necessary to identify the problem. If no sample is taken and stored, it becomes impossible to find out if subsequent infections were due to contamination by a previous contaminant, or if they were caused independently. The proper method of storage for contaminated sample is generally the same as for other samples taken from the process.

Bacterial and fungal contamination of bioprocesses are relatively easy to prevent, and thus the best strategy is usually based on prevention and eradication of contamination if it does occur. This is also true for viral contaminants, but there is an additional factor to consider. This is most common in the dairy industry and depends on an acceptance of the presence of contamination in the facility. The production and use of phage resistant strains, preferentially in cocktails of different strains with different phage resistance, means that it is possible to obtain a product, even in a heavily contaminated environment, but it requires a constant change of production starter cultures, as phages overcoming strain

resistance would be constantly selected. In the case of the dairy industry, the main problem is the contamination of raw material, which always consists of bacteriophages. However, the phages contaminating dairy industry facilities are also the ones to blame for the majority of production failures.

CONCLUSION

Contaminations in bioprocesses can cause very serious problems, but there are methods to decrease their frequency or even prevent their occurrence. Crucially, the most important point is that when contamination occurs, every effort must be made to stop its spread in the facility. This often requires rapid detection methods or skilled personnel capable of recognising the problem. For the best results in preventing contaminations in bioprocesses, make sure preventive measures are taken in the early stages of facility planning. However, remember the situation can be improved at any stage, even in already functioning facilities, for example by changing some of the work practices or including proper emergency procedures.

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