Microbial Contamination Prevention and Quality Management in the Manufacture of Agricultural Biologicals

Guidelines and Best Practices

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Acknowledgments

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Foreword

Dear Reader,

One of the big challenges facing us today, as well as in the future, is feeding our growing world population. This requires sustainable agriculture which in turn depends on the modern tools and technologies provided by plant science. One of the essential tools are crop protection products (CPPs) and these can only deliver the desired results if the high quality of the CPPs is ensured.

The commitment of the CropLife International Operations Committee is to ensure our member companies, with our external manufacturers, implement the contamination prevention systems at all phases of the manufacturing and supply chain process to prevent contamination incidents.

All of us must make sure the awareness of the importance of contamination prevention in biologicals is maintained at a high level and at all stages in the crop protection industry.

On behalf of the CropLife International Operations Committee, I hope you will find that this new edition will help you yet further with your commitment to prevent contamination incidents and to further improve the quality of biologicals.

Dirk Backhaus

Chair, CropLife International Operations Committee

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1 Introduction

The use of agricultural biologicals (ABs) for disease control, insect management, improved yield, and quality of products in agriculture, horticulture, vector control and so on is growing rapidly. Over the past two decades, a range of new agricultural biologicals have reached the market. These products, derived from biological processes, are increasingly applied to replace, or complement, several classical chemical crop protection products.

This growing demand of ABs for crop protection means an increased need for production at an industrial scale, and in response to this trend, several existing facilities have been expanded, and new facilities are being constructed.

There are major differences between the production of ABs and classical agrichemicals, and this may require different management practices to ensure consistently reproducible product quality and prevention of potential microbial contamination issues.

The risk of microbial contamination in the biotechnological processes used in the manufacture of biologicals is significant and needs to be addressed accordingly.

The emphasis in this booklet will be focused specifically on microbial contamination prevention and best practices in all operational steps in the production of those agricultural biologicals. The guidelines are globally applicable to all CropLife International member companies, and to their current and future external manufacturers.

2 Purpose and Scope

The purpose of this booklet is to provide guidance on microbial contamination prevention and quality aspects to all manufacturers of biologicals for agricultural, horticultural, and related purposes. The products which are considered in this booklet are all based on fermentation processes.

The guidelines are applicable to operational aspects, e.g. the purchase and storage of raw materials, fermentation processes, product recovery, formulation, packaging materials, filling, sterilization and cleaning, warehousing, and transport of finished product, as well as all laboratory activities. Microbial test methods, cleaning procedures and the optimized choice of disinfection products will be discussed. Legal aspects related to the quality of biologicals are also covered.

AB products, as defined in these guidelines, are products that are derived from living organisms. They may contain a living micro-organism as an active substance, including lower fungi and viruses, may be cellular or non-cellular in nature (e.g. cellular extracts), and may be capable of replication or of transferring genetic material. In some cases, AB products may only contain specific non-living components isolated from the micro-organism, e.g. products produced from a living organism, but no longer containing the whole living organism such as small molecule natural products.

Excluded from the scope of this document are:

- Biologicals used in human or veterinary medicine:
    - The production of those products is subject to different legislation and requires implementation of GMP.
- Macrobials (macro-organisms):
    - Entomopathogenic nematodes
    - Insects and mites, e.g. predatory wasps, pollinators (bumblebees), predatory mites.

- Biochemicals/Botanicals (e.g. plant extracts and essential oils) and semio-chemicals, pheromones.
- Biostimulants based on humic acids, fulvic acids, protein hydrolysates and amino acids, seaweed extracts.
- Genetically derived crop protection products such as those created using fermentation to produce e.g. mRNA products.

These guidelines are globally applicable to all CropLife International member companies, and to their current and future EMs (external manufacturers). Contamination prevention and other quality standards should form part of contract manufacturing / distribution (e.g. transport, storage conditions) of all contract manufacturing / transport / distribution agreements with EMs / distributors to ensure the required product quality is achieved and maintained.

This document does not replace the guidelines aimed at the prevention of microbial and chemical contamination issued by CropLife International: "Prevention and Control of Microbiological Contamination in Crop Protection Products" and "Contamination Prevention in the Manufacture of Crop Protection Products." Where appropriate these guidelines must be implemented in parallel.

Biological products are differentiated from chemical products because they are typically produced by a fermentation process. While not solely a concern for biologicals, microbial contamination can be a larger risk to biological processes and products compared to traditional chemical products. Broadly, microbial contamination is the presence of any undesired microorganism.

Microbial contamination of final products can have significant business and operational consequences. The product type, formulation, production process, type of microbial contamination, and application can impact the product performance. Therefore, the risk and impact of microbial contamination is product and process specific and should be assessed on a case-by-case basis.

Regardless, the following are possible consequences of microbial contamination and contamination in general:

- Supply shortfall and Force Majeure
- Loss of raw materials, increased waste costs, additional costs associated with rework (if possible)
- Loss of sales / revenue
- Applying resources to investigation, troubleshooting, cleaning, returning to normal operations
- Product and/or environmental regulatory compliance violations
- Unintended crop damage or ineffective product
- Negative customer confidence and company reputation.

3 Requirements and Responsibilities

The intent of this chapter is to outline the measures that will help reduce the risks of microbial contamination while helping to ensure compliance with the legal requirements associated with the manufacture of agricultural biologicals.

All CropLife International member companies, their external manufacturers (EM) and distributors shall commit to the requirements in this chapter.

Avoiding microbial contamination helps to ensure that the products on the market do not contain microorganisms and residual impurities not defined in the product specification, at levels which will prejudice safety, quality, and efficacy, or which do not meet regulatory requirements.

3.1 General Requirements

- Put in place documented risk assessments of microbial contamination prevention and quality standards for all raw materials, including packaging materials
- Define and document acceptable levels of contaminant in finished products
- Ensure effective cleaning, sanitizing and sterilization procedures in place as appropriate
- Ensure an effective program is in place for good personnel and plant hygiene
- Ensure validated analytical methods are available to determine microbial contamination in final product matrices.

3.2 Management Responsibilities

- The management of all CropLife International member companies and their EMs must accept the following responsibilities and shall ensure all related requirements are met to ensure:
- Adequate resources are available for all aspects of biologicals manufacturing
- Appropriate manufacturing and lab facility design, construction, and maintenance are in place
- Maintain compliance with all applicable regulations
- Skillset of employees are appropriate and necessary training programs are in place
- Able to address aspects of Biosafety (i.e. Biosafety committee)
- Requirements, and best practices are considered
- Effective quality management process is in place
- Individual identified who is responsible for quality and addressing contamination issues.

3.3 External Manufacturers Responsibilities

It is expected that the items below are incorporated in the agreement / contract between the member company and the EM. There may be additional specific requirements agreed between client and the EM.

The member company responsibilities:

- Company should share known existing risks of contamination including known-hot spots within the process and typical levels of CFU
- Company should share best practices or existing microbial testing methods
- Ensure contract specifies financial responsibilities for supplies and equipment for testing
- Undertake detailed site audits and other due diligence activities (including the cleaning process and results) and support the EM where appropriate.

The EM responsibilities:

- Communicate all contamination events to the company
- Track materials and retain all relevant records as defined by the company to enable traceability
- Obtain approval from the company prior to any change that impacts the risk of contamination and/or the quality of the finished product.
- Ensure the retention time and storage conditions of retained samples
- Evaluate the efficiency of the sanitizing and sterilization processes.

3.4 Responsibilities with suppliers of Raw Materials, Intermediates, and Strains

Products purchased from suppliers of strains, intermediates and/or raw materials must meet all quality criteria, including those related to prevention and/or control of microbial contamination.

Protection of a supplier's intellectual property remains important. Therefore, it is strongly recommended to implement a secrecy agreement. The business partners should agree in the contract to implement all requirements listed in this booklet.

As a minimum the following aspects related to microbial contamination should be covered in the supply contract:

- Definition of microbial contamination and contamination prevention (as described in this booklet; see also the Glossary)
- Acknowledge the risk for microbial contamination with respect to raw materials and their use in the process and assess if additional requirements/ specifications need be applied
- Agreement of microbial contamination specifications including a list of limits and the methods used for quantification on the certificate of analysis
- Requirements for notification of process changes as required

These aspects are additional to, and complement, the requirements laid out in the "Contamination Prevention in the Manufacture of Crop Protection Products, Guidelines and Best Practices" booklet.

4 Legal Aspects

4.1 Introduction

The purpose of this chapter is to review regulatory and other government / local requirements of Agricultural Biologicals (ABs). The comments pertain only to ABs used as bio-pesticides, bio-stimulants, and bio-fertilizers.

Many ABs have often a narrow(er) host range and target specific pests. Therefore, they may show only limited non-target effects and have minimal adverse effects on humans and the environment. ABs often also degrade more readily in the environment than some synthetic pesticides and may therefore display minimal long-term ecological effects. So, they often have an inherent lower risk than some synthetic products and therefore, this makes them a desirable option.

However, it would be erroneous to assume that all ABs are automatically safer than synthetic pesticides. Therefore, the inherent risks of the use of the different ABs must be fully evaluated.

4.2 Regulatory

In most countries and geographies, regulatory requirements for ABs have, to a varying degree, been incorporated in existing legislation covering registration of crop protection products and fertilizers.

A short overview based on current information available will be given below for several countries/geographies. It should also be pointed out that currently many authorities are reviewing and updating their guidelines and therefore it may be necessary to regularly consult and update existing databases.

- **United States**
  The evaluation of registration applications of pesticides is conducted by the EPA under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). ABs (and other Biopesticides) are considered in a separate regulatory pathway compared to conventional chemicals. Depending on the product, these are often classified as “reduced risk pesticides,” i.e. of a lower risk than conventional. This could result in a reduction of required data.

- **Canada**
  The Canadian data requirements can be found in the *Regulatory Directive: Guidelines for the Registration of Microbial Pest Control Agents* published on 30 March 2001 (DIR2001-02), Biostimulants/ biofertilizers are not regulated under PMRA. The Canadian data requirements are essentially harmonized with those of the United States (U.S.) Environmental Protection Agency (EPA).

- **Australia**
  The APVMA (Australian Pesticide and Veterinary Medicines Authority) mentions in their guidelines (Version 2, updated 14th February 2018) that differences in the characteristics of ABs and traditional crop protection products warrant the determination of the data requirements for ABs be on a case-by-case basis. Bio-stimulants including bio-fertilizers (i.e., not used for pest control or specific growth regulation) will not need to be registered by APVMA. However, these are covered under State/Territory fertilizer regulations.

- **South Africa**
  The guidelines on the specific data requirements for the registration of biologicals have been updated in June 2015 by the South African department of Agriculture, Forestry and Fisheries. These must be used in conjunction with the relevant OECD guidelines (e.g. requirements of toxicology

studies). Also falling under these guidelines are legume and other inoculants, and biological fertilizers. Specific requirements apply for the latter two categories.

- **European Union**

  The procedure for registration of biologicals in the EU is outlined in Regulation (EC) 1107/2009 which supersedes Council Directive 79/117/EEC and Council Directive 91/414/EEC. This regulation applies to both traditional crop protection products and biocides. Before revision of 1107/2009, ABs would also fall under this regulation.

  - EU 2022/1441 Commission Regulation provides which information needs to be provided to support registration.

  Bio-stimulants that can be considered as "fertilizing products," (e.g. enhance plant nutrient use efficacy, improve quality traits, increase the availability of confined nutrients in the soil etc.) must now meet the criteria outlined in Regulation (EU) 2019/1009.

- **Brazil**

  The regulatory requirements for biopesticides show many similarities with those implemented by US EPA. The definitions used for the various types of biopesticides are virtually identical. The registration for ABs used as plant protection products is granted by MAPA (Min. of Agriculture, Livestock and Food Supply) after approval by the health authorities (ANVISA) and the Brazilian Institute for the Environment and Natural Resources (IBAMA). In the case of biologicals with non-agricultural applications (e.g. veterinary), IBAMA is the registration authority. Biostimulants and Biofertilizers are regulated by MAPA and the individual states.

- **Argentina**

  The registration process of ABs is coordinated by SENASA (Servicio nacional de Sanidad Agraria) in cooperation with three other authorities: COSAVE, SACPyA and ANMAT.

  There is additional information at *https://www.fao.org/pesticide-registration-toolkit*

  This document also provides information on registration procedures and requirements in other Latin American and Caribbean countries.

- **Asia**

  Up -to-date information on registration requirements of ABs in Asia can be found by consulting: *https://www.fao.org/pesticide-registration-toolkit*

4.3 Harmonization of Requirements and Recommendations

As early as 1988, the FAO created "Guidelines on the registration of Biological Pest Control Agents" focusing on microbials and biochemicals (e.g., semio chemicals).

This document was superseded in 2017 (see below) when the FAO and WHO published jointly the following document: "International Code of Conduct on Pesticide Management, Guidelines for the registration of microbial, botanical and semio-chemical pest control agents for plant protection and public health uses".

OECD established in 1999 the Biopesticides Steering Group with the objective to facilitate harmonization of the regulatory processes. Please refer for more information under the OECD homepage:

https://www.oecd.org/chemicalsafety/pesticides-biocides/biological-pesticides.htm

5 Quality, Environmental, Health and Safety Standards (QEHS Standards)

5.1 Responsible Care®

CropLife International companies, their global affiliates, EMs, and service partners voluntary and strictly adhere to the principles described in the Responsible Care Global Charter manufacture high quality agricultural biologicals (ABs). Several Responsible Care aspects, with focus on ABs, will be further highlighted below. Appropriate management systems covering, for example, all the different aspects of procurement, manufacturing, cleaning, and sanitization processes must be in place to meet these standards**.**

5.2 Occupational Health and Safety

The health and safety of employees and contractors in CropLife International member facilities and those of their EMs, the neighbors living near the facilities and the users of the ABs is a topic that deserves a very high level of attention. It shall be a major component of the QEHS programs of the member companies, and it is expected to be of great interest to all relevant regulatory and governing authorities. It is to be expected that such authorities will carry out independent evaluations of all aspects related to occupational health and safety. As such, data on Occupational Exposure to microorganisms during manufacturing and laboratory procedures, formulation, packaging, transport, handling, use and disposal should be maintained and readily available to allow risk assessments.

To register a microbial pesticide, the micro-organism must be fully characterized, and the appropriate Environmental and Toxicological tests must be performed. Important to note is that microorganisms are classed in the Risk groups (RG) /Biosafety levels (BSL). These classifications may not specifically address any sensitizing or toxic effect caused by, for example, bacterial spores, fungal spores, or microbial metabolites.

Exposure hazards to workers can include immediate or long-term repeated exposure to fermentation by-products and culture components and must be evaluated. Other chemical or physical exposure hazards during any of the manufacturing stages must also be evaluated. Furthermore, consideration should also be given to exposure hazards of the applicators /end-users according to label information.

5.3 Risk Assessment Process

Risk assessment is an integral part of the Quality Management System. The following section provides guidance on the design and execution of an effective risk assessment process. Establishing a standardized process for Contamination Risk Assessment is critical to successful prevention of contamination incidents. Functions and organizations involved in processes and tasks that impact contamination prevention as well as all other product quality aspects in the manufacturing of ABs must have a defined risk assessment process. The process outlined below serves only as an example. The design of the process is the responsibility of the individual company.

An initial contamination risk assessment is necessary for all production units and needs to cover all products manufactured. Over time, as production units or products are changed or new ones introduced, additional risk assessments are required.

5.3.1 Design of the Contamination Risk Assessment Process

To design the contamination risk assessment process, the relevant inputs, the methodology (e.g. FMEA or Hazard Analysis and Critical Points) used, the expected outputs as well as the roles and

responsibilities must be defined. Each individual company, based on their Quality Management Systems procedures, must define all these attributes.

The risk assessment process typically includes stakeholders from Production, Quality Assurance, Quality Control, Management, and the Process Owner. It is important to note that risks that exist in manufacturing are in many cases multifactorial, i.e. there could be more than one contributing factor.

It is important that the inputs whose risk are assessed include all key factors or changes that could affect the contamination risk, such as, but not limited to:

- R&D of new formulations or changes to formulations (choice of raw materials, biocides etc)
- New product (AB) launch – introduction of new microorganism at site
- New or updated procedures or processes
- New facilities
- Equipment updates in existing facilities
- All Management of Change relevant activities
- The expected output of a completed risk assessment must include
- Go / No Go Decisions; whether to accept, mitigate, or reject the risk
- Action items, responsibilities, and timelines
- Implementation controls
- Regular risk assessment reviews and update in case of changes.

5.3.2 Process for Risk Assessment

For illustration, the assessment shown below follows the *Failure Mode and Effect Analysis (FMEA) methodology.*

Key components of an FMEA workflow are as follows:

- **Appoint a Risk Assessment Team**
  A team must be appointed to identify and evaluate the potential risks associated with the process under investigation. This should include the relevant stakeholders in the process.

- **Identification of Potential Failures**
  Identifying potential failure modes (inputs for FMEA) is a systematic, proactive way to evaluate the source and impact of failures. The potential failure modes must be documented, for example in a **Q**uality **R**isk **A**ssessment **T**emplate (QRAT).

- **Analyze and Ranking of Potential Failures**
  Severity, **O**ccurrence and **D**etection (SOD) values provide a tool to rank potential failures based on three criteria: the severity, the likelihood of occurrence and the potential detection of a defined scenario. Values are assigned based on pre-determined criteria.

- **Calculate and Evaluate Risk**
  The rating of the risk needs to be translated into a tangible scale and classified by pre-determined acceptance levels. The **R**isk **P**riority **N**umber (RPN) obtained by multiplication of the SOD values allows us to estimate the level of risk. The RPN indicates whether additional measures are necessary, or a risk is acceptable, based on the previously defined acceptance levels.

- **Identify Risk Reduction Measures**
  For risks not meeting the threshold of the defined acceptance level, adequate measures need to be developed to reduce the risk to the required acceptable level.

- **Evaluation of the Effectiveness of Proposed Measures**
  Before the implementation of any risk mitigation measures, it must be ensured that they will lead to the intended result. The risk is re-evaluated considering the proposed measures and whether the defined threshold will be achieved. If this re-evaluation indicates success, the defined measures can be implemented. If not, other measures need to be identified.

- **Documentation**
  The risk assessment, all corrective measures and their approvals must be documented and controlled.

5.4 Contamination Response

If a contamination is detected, an emergency procedure or decontamination plan should be initiated. This procedure should include at least the following actions:

- Assess the need to isolate further contamination downstream if required
- Place potential effected internal and external inventory in quarantine, until the scope of the contamination is understood
- Ensure samples are taken from all possible sources of the contamination such as raw material inputs to facilitate the investigation of root cause
- Extended cleaning and sterilization procedure (emergency response in place)
- Extended microbial monitoring to check the effectiveness of the preventive action taken – e.g. Microbial assays, environmental swabs, or media simulation (sterility run with pure media without bacterial culture).
-

Following a contamination event, it is important to initiate a formal contamination investigation immediately thereafter. The purpose of this investigation is to attempt to identify the root cause of the contamination event and identify corrective actions that will be taken to prevent or reduce the likelihood of future contaminations. It can be very difficult to identify the root cause in many contaminations, however even in these cases the investigation frequently identifies changes or steps that can be made which will reduce the risk of contaminations in the future. The contamination investigation should include several individuals who have detailed knowledge of the equipment and process where the contamination occurred as well as those with expertise in aseptic processing.

Partnering with an industrial hygiene expert with expertise in contamination treatment and prevention can be very valuable in both identifying potential root causes and implementing corrective actions and improvements to the cleaning program.

6 Overview of Contamination Sources

Microorganisms including spores and phages are ubiquitous in nature. In addition, microorganisms can adapt and survive under a variety of conditions. These aspects make them a significant risk to biologic products. An understanding of the microbial entry points and implementation of measures to prevent microbial contamination is critical for the manufacture of biologic products.

As shown in Figure 1 and detailed in table 1, microorganisms can enter a production process stream from several sources. All sources of microbial contamination should be considered when developing a microbial control strategy and performing an investigation for a microbial contamination deviation.

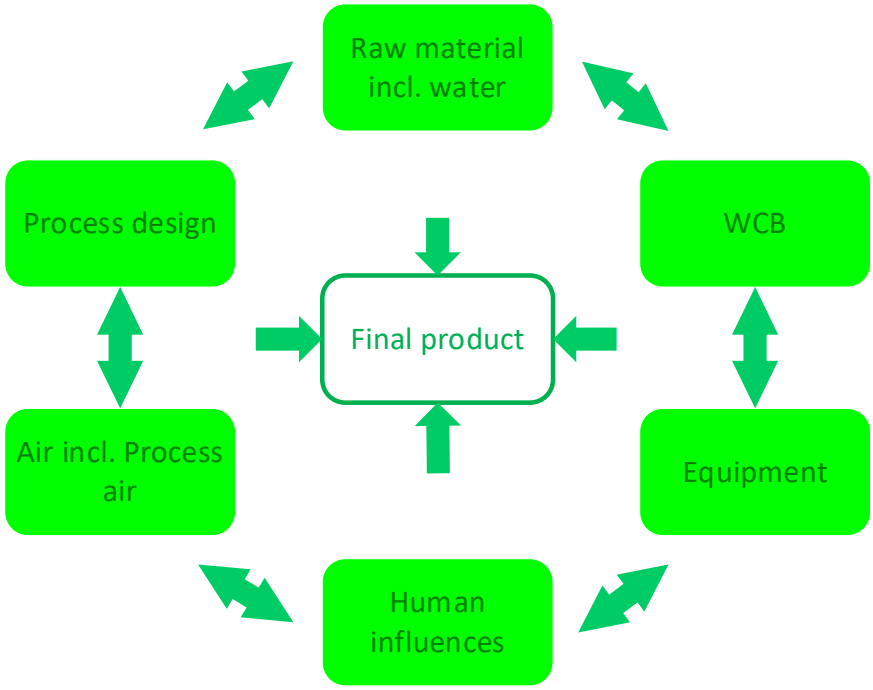


Figure 1: Sources of contamination in processing plants

Source	Details
Raw material including water	Insufficient media sterilization Contaminated raw material Quality of water
WCB	Microbial and Phage contamination Heterogeneous (mixed) culture
Equipment	Facility design, inadequate equipment, design Use of contaminated water for cleaning of equipment Insufficient steam quality or cold spots in piping / vessels / add-on parts Pressure failure (contaminant intrusion caused by vacuum) Gasket materials - leakage / crack Cross-contamination / ineffective bioreactor decontamination Ineffective cleaning (esp. valves, crevices, sampling ports and “dead ends”) Biofilms on equipment Surface contamination Steam traps not working correctly Too long maintenance cycles (O-rings, valves, filter) Low maintenance of clean benches Improper operation of feed sterilization system Failure of liquid sterilizing filter or use on inadequate filter media
Human influences	Contact / movement of employees Imprecise SOP / documentations Insufficient training / hygiene Poor management of change
Air incl. Process Air or Nitrogen	External and internal pollution (airborne phages, dust particles and fluid droplets) Insufficient inlet gas filtering: (Membrane failure, insufficient pore size, air moisture, improper sterilization, excessive usage –> deterioration) Insufficient decontamination of fermenter exhaust gas Avoid backflow (contaminated air etc.)
Process design	Aerosol sources (personnel, air ventilation, air conditioning, high-pressure cleaneretc.) Improper design or usage of inoculation systems Location of waste treatment, sewage Improper design or usage of feeding system especially shared feeds Improper design or usage of sampling systems Area design, movement concepts and access control

Table 1: Routes of contamination from various sources

7 Human Influences

Employees, visitors, maintenance people, contractors and logistics personnel harbor a potential source of microbial cross contamination, especially if they are moving from one processing area to another. Tools or equipment they use could also lead to additional contamination.

Personal Hygiene Practice

Personal hygiene policies and procedures should be implemented by all personnel at each company and in each facility to reduce the risk of product contamination.

Cleanliness and Attire

Protective clothing is a barrier between the operator and the product. Their purpose is to protect the product from the person, not the person from the product. These items typically include company-provided coats (e.g. lint-free, no outside pockets, plastic aprons or sleeves, hair nets, gloves, and footwear when appropriate.)

The protective clothing:

- Must always be worn, should be dedicated specific lab or production areas and should never be worn outside of these areas
- Must be appropriate for the area and the type of work performed there. Specific regulations or requirements must be followed
- Must be regularly changed
- Must be cleaned and sanitized adequately (controlled by the company where required)
- Should be color-coded or otherwise distinct to make it obvious to all employees who is and isn't allowed to access specific areas
- Additional clothing may be required in QC laboratories handling MCB, and WCB in a controlled environment.

Personnel Hygiene

An effective hand washing program must be implemented ("how to wash hands"). Therefore, an appropriate number of appropriate hand-washing resources is necessary. These should be placed at convenient locations and proper operation and use verified on a regular basis.

Employees should be clean and follow industry standard requirements and area specific regulations regarding personal belongings (e.g. using jewellery, common nail polish, fragrance, food consumption).

Training

An effective and continuously improving training program is essential and should include components appropriate for all levels of people and their roles in a plant: the personnel (management, operators, sanitization, and maintenance staff) as well as contractors, temporary staff, and visitors. The training should include various topics, many found in this booklet, such as personal hygiene, cleaning and disinfection procedures, process handling and testing. Periodic refresher training and testing should

be part of the overall training program. An immediate training on personal hygiene policies and practices for new employees should be planned.

8 Facility Design and Engineering Recommendations

Biological processes have some unique requirements on the design and operation of the facilities where they are implemented.

8.1 Differences between Chemical and Biological Processing Plants

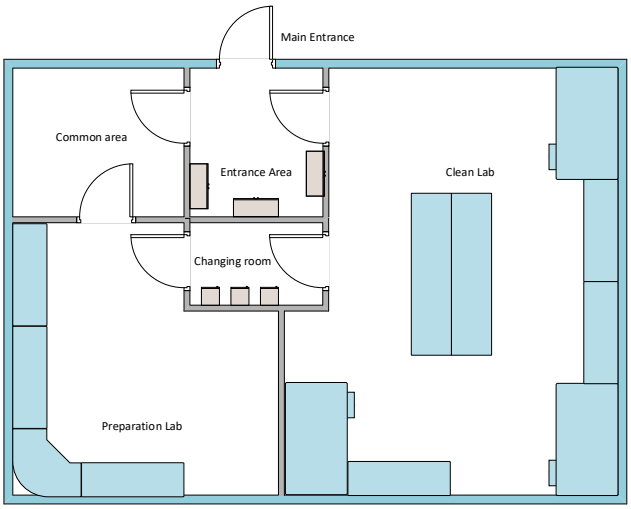
The variability in biologic systems is much higher than in chemical systems. Biologic processes include living systems with intrinsic variabilities, undefined or poorly defined raw materials, and process-induced variabilities (e.g. fermentation conditions, cleaning).

Topic	Chemical	Biologic
Process	Chemical synthesis	Fermentation
Substrate	Specific chemical ingredients	Complex natural media, use of living systems
Conditions	High temperatures, high pressures	Mild conditions, physiological pH, and temperatures
Concentrations	High	Lower
Solvents	Mostly organic	Mostly water
Process robustness	Lower variability	High dependency of raw materials and process conditions
Conversion rate	Up to 100 %	30-70%
Reaction time	Hours	Days
Products	Well-defined chemical structures, often single molecules, low molecular weight compounds	Often complex mixtures of high molecular weight compounds or whole micro-organisms, (side) products depend highly on raw materials and process conditions
Analytic	In most cases complete analytics available	Often the final product cannot be fully characterized, the analytical tools are limited and time consuming
Cleaning	Toxic wastewater	Disinfection/ sterilization needed
Waste	Mostly toxic	Not toxic, high organic load
Main risks	Process/ operator safety	Microbial contamination
Hygienic concept	Larger toolbox for control	Mandatory

Table 2: Main differences between chemical and biological processes

8.2 Lab Design

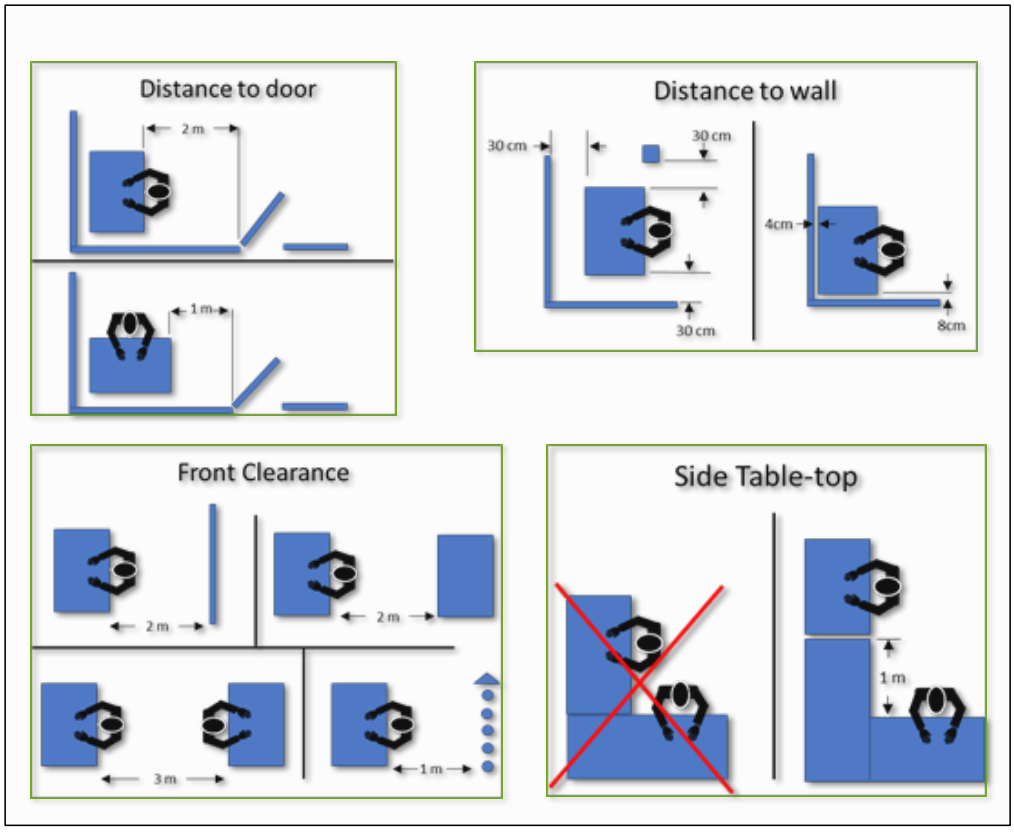
Building and room air is most critical for the laboratory phases of the production process. It is common that the strain scale-up of the Working Cell Bank (WCB) occurs in classified clean rooms or clean areas. Based on risk, air filtration, air flow direction and air pressurization can be used to control particulates as appropriate for the type of activities in the area (increased control in higher level hygiene areas). See example:



8.2.1 Position of Laminar Flow Bench in Laboratories

Biological safety cabinets (BSC) must be installed so fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled areas, and other possible airflow disruptions.

Examples for the position of Bio Safety cabinets



8.3 Construction, Design, and Lay-out of Production Units

The plant and process design are important to guarantee product quality and to minimize the risk of contamination.

Manufacturing plants of biological crop protection products are normally not designed to operate entirely under strict sterile conditions as a biopharmaceutical plant would. Consideration must be given to where sterile conditions are absolutely required and where it is sufficient to minimize the introduction of microbial contaminants based on the product and process being used.

Nevertheless, a comprehensive and detailed approach to plant and equipment design and selection is required to ensure that the products produced will meet microbial contamination limits. This should include not just aspects related to times of operation but also to ease and effectiveness of cleaning between uses.

8.3.1 Facilities, Utilities and Supporting Functions

- In biologicals processing, contamination detection and prevention are essential throughout the main production line from the working cell bank culture to the final packaged product. However, the systems supporting or supplying material to the main production process can also significantly impact sterilization, cleaning, and therefore contamination risk.
- The following support systems are typical for a biological process:
- Waste treatment
- Important considerations for waste handling include proper waste classification, movement and storage of liquid and solid waste, wastewater treatment requirements, post-treatment disposal and compliance with local regulations.
- Unique considerations for waste from a biologicals process relate to preventing contamination of the production process, uncontrolled release of the microbial active material to the environment, and characteristics of biologically generated waste compared to other sources of waste.
- Typically, waste streams in a biologicals facility are not sterile; therefore, the proximity, transportation, and handling of waste should be considered relative to sterile or axenic systems to minimize cross-contamination risk.
- The risk of an uncontrolled release of the production strain should also be evaluated. Best practice is to ensure live production strain does not leave the process in waste or vent streams. The volume of fermentation-based waste relative to the capacity of municipal or other treatment facilities should be considered. Additional on-site treatment systems may be necessary to comply with environmental regulations.
- Adequate supply of air
- Biotechnology plants need large volumes of clean, dry, oil-free air to support cell growth during aerobic fermentations.
- Ventilation and air conditioning
- Air quality is critical for the laboratory phase of the production process. It is common that the strain scale-up of the WCB occurs in classified clean rooms or clean areas. Typically, this involves air filtration and high air exchange rates to control particulates and certain rooms are positively pressurized relative to neighboring spaces to minimize air flow into the clean areas

from connecting spaces. If the area is intended to work with higher risk classification organisms, more sophisticated monitoring, and controls of the air system in the area may be warranted.

- Humidity and temperature controls should be considered and optimized for the process.
- In an enclosed fermentation area or a room containing autoclave equipment, efficient ventilation or cooling should be designed to remove heat from the building during the sterilization process.
- Inlet screens and coarse filters can be used to prevent entry of pests and environmental debris.
- Dust collector
- Used for plant hygiene to collect dust created during handling of raw materials or transfer and packaging of some products.
- Exhaust Treatment
- Exhaust from the fermentation process is typically treated for odor control, environmental compliance, contamination prevention, and preventing the uncontrolled release of the production strain.
- Exhaust treatment methods include but are not limited to chemical scrubbing, filtration, and thermal treatment (e.g., flare).
- **Steam**
  For locations where steam contacts the process, the steam must be of high enough quality, so it does not introduce chemical or microbial contaminants.

8.3.2 Facility Layout and Workflow

Depending on the level of microbial contamination potential, some form of physical separation often needs to be implemented. Common examples include the physical separation between WCB storage, laboratories, process rooms, and waste treatment. Air handling systems should be set up to establish pressure differential between rooms as necessary to prevent flow of contaminants, especially to rooms with open handling (e.g., clean bench or flask operations).

Physical separation considerations may include:

- Identifying where dust generation or accumulation may occur especially during operation but also at rest e.g. media preparation.
- Identifying where storage of certain materials or equipment must be kept separate from one another. In some cases, people traffic between these rooms may not be permitted without a change of clothing, shoes, etc.
- Determining what is the appropriate level of separation of operations to avoid cross contamination.
- Identifying what materials are compatible or incompatible.
- Determining whether storage of compatible materials and products can occur in a common area in the same building.
- Defining material flow of raw materials, product, waste, and samples/retain samples to minimize the risk of contamination.

- Establishing traffic patterns for product and personnel to avoid unnecessary human traffic between areas.
- Defining required level of cleanliness for each area
- Process and personal should flow optimizing movement from cleaner to less clean areas.
- Implementing color coding: For easy identification the rooms should have color codes and personnel should wear differently colored protective clothing for different operations (e.g. white in the clean and blue in the unclean operations.)
- Implementing status labeling: status labeling of equipment and rooms to denote status such as "in use", "contaminated", dirty/to be cleaned" or "clean".

The degree of hygiene control in the facility depends on the type of the facility, operation, and the analysis of the potential risk. Based on the assessment, the facility is divided into areas with different allowable processing steps, different rules and/or procedures for persons who are allowed entry, and/or different levels of cleanliness.

Generally, the more sensitive the product, the more important it is to separate the facility into different hygiene areas. Each manufacturing operation requires an appropriate environmental cleanliness level to minimize risks of contamination. Buffer zones, sanitizing stations, physical (or other) barriers are often placed between the basic hygiene areas to the high hygiene areas.

Area	Characteristics	Requirements
Raw material storage	Raw materials are not sterile, therefore are a source of contaminants.	Follow company standards
Laboratories	Preparation for production: The first steps of production are performed at lab scale. The inoculant from the WCB is the base for all production steps. Raw material acceptance, in-process sample analysis, QC of final product	Appropriate BSL, E.g., biosafety cabinets, room classification autoclaves, single use utensils. Restricted access
Fermentation area	Staging of raw material, sterilization, production of active components, sterile /axenic processing.	Aseptic design, adequate cleaning, sanitization and sterilization capabilities Restricted access
Product recovery	Recovery of active ingredient from fermentation broth, processing, and standardization of bulk	Aseptic design, adequate cleaning, sanitization and sterilization capabilities Restricted access
Formulation, Filling and Packaging	Depending on the process sterile or axenic conditions and handling may apply	Aseptic design “as needed”, adequate cleaning, sanitization and sterilization capabilities Restricted access
Storage	Separated storage between raw material, packaging material and intermediates as well as finished products.	Storage conditions according to vendor specifications Follow company standards for warehousing
Waste	General process area	Follow country specific and local regulations as well as company policies

Table 3: Characteristics and Requirements of the facility

8.3.3 Equipment, Piping, Utensils and Tools

Equipment (includes fixed installations), piping, utensils and tools should be non-contaminating and easy to clean.

There are guidance documents (e.g., ISO, DIN) for equipment recommendations. Organizations in this area are for example: the European Hygienic Engineering & Design Group (EHEDG), 3-A Sanitary Standards, Inc. (3-A SSI) and American Society of Mechanical Engineers: Bioprocessing Equipment (ASME BPE).

Product contacting equipment should fulfill the following aseptic design principles:

- Self-emptying or self-draining
- Easily cleanable (design and material)
- Sterilizable (in the case of fermenters and feed tanks / lines to fermenters)
- Compatible with product, cleaning and disinfecting agents, process, and sanitization conditions

- No use of non-reactive polymer (plastic) for tanks, pipes, hoses, valves, or fittings
- Sampling equipment and points must be sterilizable
- Surfaces should be polished, non-porous, free of scratches and cracks
- No sharp angles in tanks and piping
- Comply with aseptic welding standards (e.g., ASME BPE)
- Product contact surfaces should be accessible for cleaning and inspections
- Avoid dead legs (Residual product or materials in dead legs could support bacterial growth (e.g., instrument ports, blinded pipes, T-fitting, pumps, valves).

Criteria for hygienic design are particularly important for equipment used in the later stages of processing and particularly after a sterilization processing step.

Product contact materials that should be avoided include wood, cast iron, brass and galvanized metals. One preferred material is high quality stainless steel - if possible polished.

When considering facility design the production process may involve extremes of temperature, abundant use of water, development of condensates and contamination of product from overhead pipes and surfaces. Equipment design must consider this and include proper protection, e.g. for steam sterilization incorporate steam traps in production line. Surfaces become hot during sterilization and should be properly insulated or guarded to prevent personnel exposure.

8.4 Maintenance

Maintaining the process equipment is critical to maintaining plant performance and quality product. The following items are recommendations for a biological process:

- Internal and contracted maintenance workers should be trained and familiar with the plant hygiene requirements.
- Maintenance employees should document, and track tools & supplies taken into and out of controlled production areas.
- Equipment that is removed from the process for repair or maintenance should have documented cleaning protocols that are followed prior to reinstalling. In general, any product contact surface should be cleaned and sanitized prior to return to operation.
- Process contacting tools should be cleaned before and after use. Tools and equipment should not be placed on the ground or floor prior to use. Determine if certain tools need to be dedicated to specific process areas.
- Aseptic service equipment must be sterilized or sanitized before use in the process. This could be sterilization/sanitization outside the process or in-process sterilization/sanitization before return to operations.
- If possible, equipment maintained out of place should be cleaned prior to removal and tagged accordingly. Otherwise, cleaning should occur before significant work in the shop to minimize contamination spread or chemical cross-contamination.
- Maintenance programs should document and define material compatibility. This includes but is not limited to soft parts such as gaskets and O-rings in addition to piping and vessel materials of construction.

- Parts and tools should be clearly labelled and organized to ensure workers use the correct materials when executing process maintenance.
- Internal equipment and parts specifications is a way to document what is required for successful maintenance. Additionally, a preferred vendor system can ensure parts and materials consistency and prevent use of unapproved or incompatible parts, materials, or tools.
- Common maintenance activities should have documented procedures to ensure the objective is consistently completed.
- Facilities should have a documented and scheduled preventative maintenance program.

8.5 General Pest Control of the Site

Pests bring in and spread contamination (e.g. birds, rodents, roaches). A pest control plan should be in place to reduce external sources of contamination. External providers can be a good source towards this service.

Fumigation may be a last resort to eliminate bioburden. Prior to fumigation a risk assessment must be done, and all necessary safety precautions must be in place.

For biological products that are organic certified, the pesticides/rodenticides used for control must comply with the organic certifying body's requirements.

9 Manufacturing Processes

9.1 General Description of Manufacturing Processes

The production of biologicals includes several process steps or unit operations. In general, the steps from media preparation to inoculation to fermentation are referred to as the upstream process. The downstream process refers to the steps used to recover the product and may include operations like separation, concentration, and purification. Risk assessment of the process is required to identify areas susceptible to contamination and therefore requiring aseptic practices. Due to the favorable conditions for the growth of contamints, the most critical portion of the process is often fermentation.

With biologicals, the finished product often cannot be fully characterized. Therefore it is critical that manufacturers understand how changes to any of the process, including the raw materials but especially the fermentation, can affect the final product and do their utmost to ensure that the process is kept substantially the same from batch to batch and over time. Failing to do so is likley to compromise product consistency, quality, and purity. A good understanding and consistancy of the process will also allow for timely detection of a microbial contamination as deviations from normal operations can often be a symptom of contamination.

Fermenter designs, operating strategies, process parameters and methodologies are diverse. In this chapter important aspects of typical manufacturing processes are discussed.

9.2 Cell Banks

The origin of any biotechnological product is the ***master cell bank*** (MCB). The MCB is an aliquot of a single pure culture of cells which generally have been prepared from the selected cell clone under defined conditions, dispensed into multiple containers and stored under defined and tightly controlled conditions (usually at -80°C or lower).

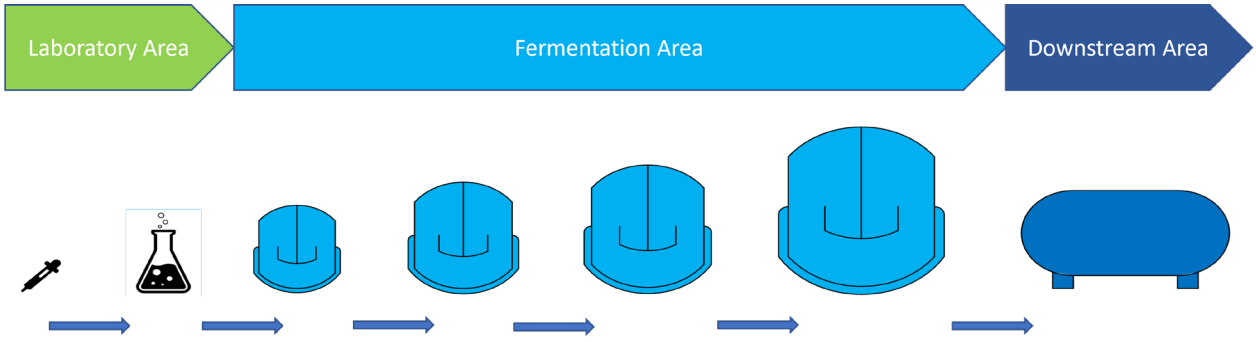
The ***working cell bank*** (WCB) is a culture of cells derived directly from the master cell bank and is intended for the regular initiation of new cell cultures for production batches. The WCB is usually stored at -80°C or lower.

Purity testing of all cell banks before use is critical to ensure absence of microbial or phage contamination. The preparation of the WCB inoculum is the first of many growth promoting steps and so maintining culture purity here is especially critical. However, since this step is typically done in a laminar flow hood and involve open handling and human interaction, this also has a high risk for contamination.

9.3 Liquid state fermentation process

The main components of a liquid fermentation are listed in Figure 2 and will be explained below. During the scale up phase from Flask Inoculum to production fermenter the contamination risk is high due to multiple transfers between vessels and the growth promoting aspect of these steps. This process should be continually monitored for microbial contamaintion and care should be taken to sterilize the transfer lines and any equipment such as receiving vessel.

The raw materials of the growth medium are mixed and sterilized directly in the fermenter or in external devices. Production fermentations are typically either batch, fed batch or continous fed batch. In the case of fed-batch fermentations, the feed solutions are sterilized in external vessels and asceptically transferred into the fermenter as needed. The addition during the fermentation creates a risk of contamination.



Working Cell Bank /Seed Culture	Seed Flask	Pre-Seed Fermenter	Seed Fermenter	Seed Fermenter	Production Fermenter	Downstream Process
Tube	Shake Flask	Glass or Steel Fermenter	Steel Fermenter	Steel Fermenter	Steel Fermenter	Steel Container
1-5 ml	1 l	10 l	100 l	1.000-10.000 l	100 m^3	10 – 100 m^3

Figure 2: Production steps from WCB through to downstream processing

9.4 Solid-State Fermentation Process

Some biologicals products cannot be produced using liquid fermentation processes and must instead use a solid-state fermentation process. There are also some products where solid-state fermentation may offer cost and efficiency advantages over liquid fermentation. Many of the stages used in a solid-state fermentation process are similar to a liquid process but the conditions, equipment involved and how each stage is performed can be significantly different.

Bases on these differences the risk for contamination also changes. The table below shows a comparison of these different risks.

Stage	Solid Substrate Fermentation	Liquid Fermentation
Inoculum production	Similar risk	Similar risk
Raw material sterilization	Higher risk due to challenges to fully sterilize solid substrate matrix	Low risk due to easier and more effective sterilization of liquid raw materials
Main Fermentation/ Incubation stage	Higher risk due to multiple smaller incubation units and longer average incubation times Risk can be mitigated by control of substrate moisture	Lower risk due to easier and more effective sterilization of liquids in fermentation vessels and average shorter incubation times
Downstream processing	Lower risk due to reduced moisture	Higher risk due to higher water content and increased open processing, like spore separation via flotation, drying, fill and pack operations Risk can be mitigated by good hygiene and timely separation and drying after fermentation.

Table 4: Solid state fermentation vs Liquid fermentation

9.5 Contamination Indication during Fermentation

The goal of fermentation is to axenically grow microorganisms under controlled conditions. Deviation from known process parameters may indicate microbial contamination at an early stage. Compared to standard fermentation, higher metabolic activity may indicate the presence of an outpacing contaminant while a lower metabolic activity may indicate growth inhibition or cell death.

Key physical/ chemical parameters to monitor for microbial contamination include but are not limited to:

- temperature
- dissolved oxygen
- pH
- foam
- biomass accumulation
- morphology
- odor
- color
- viscosity

The parameters that are usually monitored on-line and often controlled are temperature, pressure, dissolved oxygen, off-gas, pH, and foam.

Fermentation vessels are temperature controlled using chilled water. Changes in demand may indicate microbial contamination. Pressure is monitored and normally controlled to maintain at least a slight positive pressure to help avoid the ingress of any contaminants. Negative pressures, relative to atmosphere, are always to be avoided.

The demand of oxygen varies during fermentation. Deviation from a typical dissolved oxygen (DO) profile may indicate contamination. If a deviation from the expected DO profile is observed, ensure probes are functioning correctly first. Dissolved oxygen probes can be unreliable especially in more challenging fermentation media, and indications of deviations from normal values should be cross checked by other methods (e.g. off-gas analysis, microscopic sample analysis, pH)

Monitoring the composition of the off-gas, especially CO_2, provides useful information about the culture such as growth rate and ongoing respiration. Changes to composition from the normal profile can indicate the presence of contamination.

Usually, fermenters are equipped with the ability to monitor and control pH however this is not required for all microorganisms and processes. In cases where pH is uncontrolled, deviation from a typical pH profile may indicate contamination. In cases where the pH is controlled by the addition of acid or base, a deviation from the typical consumption of these feeds may also indicate contamination.

The presence of foam is usually observed visually, and high levels are indicated by a conductivity sensor. High values are usually controlled by adding an antifoam agent. An increase in the amount of foam observed can be an indication of contamination. Control of foam is needed to avoid problems with the exhaust gas system. Too much foam can result in a foam-out where broth leaves the fermenter which may interfere with off-gas analysis, blind the exhaust filter, or can lead to contamination. Besides the afore mentioned on-line measurements, off-line measurements such as biomass measurements, microscopic evaluation and direct plating may also be performed to monitor for signs of contamination.

Biomass accumulation is typically the goal for controlled fermentation processes. Growth during fermentation usually follows a standard curve for a defined process. Differences in biomass accumulation behavior could indicate contamination together with other process parameter deviations. Typical parameters to monitor biomass accumulation are optical density (OD), dry or wet biomass.

Optical microscopic analysis could provide direct confirmation of microbial contamination. Contaminants are often indicated by deviations in morphology and motility compared to the desired organism. Additionally, it is strongly recommended during the entire fermentation process to routinely use plating methods to check for contamination. While the results of plating may not show contamination for some time, the results are invariably useful for any subsequent contamination investigation.

9.6 Downstream Process

The downstream process for fermentation is focused on recovering the desired fermentation product in a way that minimizes the loss of material or activity and avoids the introduction of chemical or biological contaminants. The downstream process typically includes unit operations like separation, concentration, purification, drying and blending. It could also involve the addition of various agents which aid in product stability or subsequent formulating. Typical downstream process equipment includes filter presses, centrifuges, spray driers and blenders.

9.7 Raw Materials Including Process Water

Raw materials are a critical component to any fermentation process and variation in their quality can

have a negative impact on the fermentation and final product. Therefore, specifications should be defined for each material depending on its usage. These specifications should consider the process, the unique requirements of biologicals and the goal of preventing chemical and biological contamination.

Examples of raw materials include labware, materials for fermentation (in liquids or solid state), intermediate bulk containers (IBCs), recovery process aids and formulation components.

9.7.1 Handling of Raw Material and Product Packaging

To avoid the introduction of microbial contamination, incoming raw materials and containers should be checked for cleanliness and sterility (as applicable).

IBCs and drums that may once have contained a product with microbial growth can serve as a source of microorganisms for the fresh product that will be stored in this container. Therefore, always use new containers.

Ensure that new pallets are heat treated (if they are wood). This prevents pests/microbials from entering facilities. Using plastic pallets could be the better option.

Dirty/used pallets can often be an important source of microbes so reusing pallets should be avoided.

To avoid contamination of end-user packaging with airborne microorganisms during storage, it is required to keep packaging material covered and protected against dust if possible before placing it on the filling line.

9.7.2 Process Water

Water is a critical raw material in the manufacture of Biologicals but can also be the source of a variety of contaminants. All sources of water may have microorganisms at different levels depending on its origin, e.g.:

- Potable / tap water
- Well water
- Rainwater
- Demineralized water

Wherever used in the process, the water quality needs to be suitable for its purpose. Water used in fermentation processes must always be sterile. In cases where water does not need to be sterile, there should still be a set of specifications which include the allowable microbial limit, Verification that the water meets this specification should be done routinely. Besides meeting the microbial limits, the water may also require chemical adjustment such as softening.

There are no fixed rules regarding the design of the water treatment facilities as it will depend on the source and the specifications set for the process water. In all cases, the source water should be monitored periodically for the level of microbial contamination to ensure the incoming bioburden does not exceed what the system was designed to handle.

9.8 Sterilization of Inputs

Raw materials are one of the main sources of contaminants; hence the need to have adequate sterilization or pasteurization of all raw materials (incl. water). There are a diverse number of sterilization methods available. The most important ones for large scale applications are sterilization

by thermal treatment (inactivation of microorganisms) and filtration (physical removal). For solids, gamma irradiation is a common practice.

Media with high solids content can be a challenge for sterilization so that sterilization procedures must be demonstrated to be effective.

Sterilization conditions, such as temperature and time, can have an impact on the efficacy of the media. Therefore, assessments should be performed to verify the sterilization procedure is effective, i.e. the media is sterile, but does not negatively impact the production.

9.8.1 Media Sterilization by Thermal Treatment

The raw materials of the fermentation medium are sterilized directly in the fermenter or in external devices. For all heat sterilization methods, adequate temperature, uniform temperature distribution and adequate retention time must be ensured.

Batch sterilization is normally conducted by sterilizing the vessel and the medium at 122–125°C for at least 15 min (usually 30–45 min). Large volumes may require longer sterilization times and the heat up and cool down times can be significant. The working pressure used should ensure saturated steam conditions. For uniform distribution the medium is agitated. The sterilization operation depends on medium composition, temperature, and time as well as the temperature distribution in the fermenter.

During continuous sterilization, medium is sterilized prior to entering the pre-sterilized empty fermenter. High Temperature Short Time (HTST) continuous sterilization is widely used in the food industry. With this method, the medium is heated via heat exchangers and held at a constant temperature for the required residence time. Continuous sterilization is conducted at a higher hold temperature (135–150 °C) and for a shorter hold time (4–15 min) compared to batch sterilization. Advantages of a HTST system are that a higher rate of spore inactivation is achieved, the breakdown of heat sensitive medium components is reduced, and a better energy efficiency is obtained.

Frequently there is a period between the completion of sterilization and use. It must be ensured that sterility can be maintained during this hold period. One important aspect of this is to allow the introduction of sterile air to avoid formation of a vacuum which could draw in contaminants.

9.8.2 Media Sterilization by Filtration

Sterile filtration is used when the medium contains heat labile liquid components and when Maillard reactions of carbohydrates must be avoided. To physically remove contaminants, filters with a pore size smaller than the contaminating microorganisms are used (general standard 0.2 micron absolute).

Where possible perform post-use or pre-use integrity testing. Sometimes two sterile filters are installed in series to minimize contamination risks. Filter sterilization of media is more expensive than thermal treatment and requires solids to be sterilized separately.

Filtration is mostly used for sterilizing homogeneous gases or liquids where a minimum concentration of bacteria is present. The effectiveness of the filter sterilization must be validated to ensure uniform performance throughout the filtration. Filtration may not be suitable for all materials (e.g. oil or water emulsions, salt solutions or containing surfactants), depending on the chemistry of the filter media, the pore size of the filters and the material to be handled. It’s important to know the material to be handled does not damage the filter.

9.8.3 Gamma Irradiation

Where thermal treatment or filtration is not suitable, such as for sterilizing solid materials, gamma irradiation can be used. In principle, the gamma irradiation of raw materials, natural solid nutrients,

inorganic materials, or thickeners is possible. It needs to be verified that the receiving country accepts gamma irradiated products.

There are country-specific requirements that must be observed when performing sterilization by gamma irradiation and so these activities are strictly handled by contracting specialized external service providers.

9.8.4 Water Sterilization

Fermentation processes, product recovery and final product preparation frequently require the addition of sterilize water. The sterile water may be produced by thermal sterilization using batch sterilization (typically at 121°C for a minimum of 30 minutes) or by continuous sterilization (135°C minimum for a minimum of 2-5 minutes). Alternatively, sterile water can be produced using filter sterilization. The latter still requires a robust method for sterilizing the filter and the connecting piping or tubing.

9.8.5 Process Air and Gases Sterilization

During the fermentation of aerobic microorganisms, a large amount of air is required. In various processes other gases may also be introduced. To sterilize gases, filtration is by far the most common method and typically it involves a two-stage process.

In the first stage a pre-filter is used to remove most of the solids including coarse dust particles. This is normally done using a depth filter which may contain polypropylene (PP) filter media. A depth filter is especially useful when the amount of solid material is relatively high, and a large solids capacity is desired. If only a small number of solids are present, a membrane filter may be chosen to better protect the stage 2 filter. Membrane filters may contain polytetrafluoroethylene (PTFE) filter media. Based on the depth filter construction type, the nominal pore size should normally be around 10 µm to remove particles down to approx. 1 µm in gases.

The second stage is the sterile filtration. Here mainly membrane filters in a cartridge configuration are used. Filter cartridges show a higher stability against defects than panel filters. The filters should be made of hydrophobic material like PTFE to prevent wetting and the pore size is typically 0.2 µm absolute. The filter is typically placed in a stainless-steel housing and can be sterilized in place with steam. A low pressure drop across the filter and its housing is generally desired for larger vessels. It should be kept in mind that the lifetime of a filter is finite and depends on sterilization and process conditions. Integrity testing is conducted where possible and when it is not, the filter should be replaced on some pre-determined frequency and when the pressure drop across the filter increases to some threshold value.

9.9 Filling and Packaging

Measures to be taken on the prevention of the contamination during filling and packing depend on the type and composition of the formulated product, the water activity and the storage conditions.

Some sensitive products may have high levels of nutrients and water content which can lead to additional growth after packaging. As such high levels of undesired microorganism must be avoided. For these sensitive products the following practices should be considered during filling and packaging to minimize the introduction of undesired microorganisms.

- Use of sterilized packaging (where required)
    - Store packaging in a clean and protected environment. Don't remove the outer packaging until ready for use.

- Sterile filling equipment
    - Depending on the equipment some parts may be made out of plastic or rubber, in this case sterilization with hot water / steam is not possible. In this case disinfection or sanitizing by chemicals is necessary.
    - Use sterile single-use parts if possible.
- Monitor contamination risk of work areas with environmental air and surface swab testing.
- Sterilize or adequately pasteurize added component (e.g. water, inerts).
- Use controlled and clean environments as appropriate
    - If the filling process requires open handling, this should be done in a filling chamber or clean room separate from the open space.
    - Using HEPA filtered air and positive pressure to reduce particles and microbial contamination.
    - During unoccupied periods consider the use of UV-C light in the filling area to reduce buildup of contamination in room and on equipment surfaces.
- Use appropriate Personel Protective Equiment
    - Protective clothing (e.g. gloves, Tyvek suits, face masks, hair covering, over-shoes, goggles) may be required during open product handling processes.
- Minimize opportunities for growth of contaminants
    - Where cold storage is required, move packed products in cold storage directly after filling.

9.10 Transport and Warehousing

Temperature and humidity control is important for Biological products due to their biological properties. This is not only for shelf life reasons, but also to prevent contamination growth in the product.

Environmental storage conditions and packaging that is suitable for the product must be determined. When required, temperature and humidity should be monitotred and controlled during transport and warehousing.

9.11 Waste Treatment and Disposal

All waste must be removed from the processing area and premises on a regular basis. For waste treatment all country and local regulations must be followed.

Unique considerations for handing of waste from a biologicals process include preventing contamination of the production process, uncontrolled release of the production strain, and characteristics of biologically generated waste compared to other sources of waste.

Typically, waste streams in a biologicals facility are not sterile. Therefore, the proximity, transportation, and handling of waste should be considered relative to sterile or axenic systems to minimize contamination risk to the latter. The uncontrolled release of the production strain should also be evaluated. Best practice is to ensure live production strain does not leave the process in waste or vent streams. Finally, fermentation broths are usually high BOD/COD streams. Volume of fermentation-based waste relative to municipal or other treatment facilities should be considered. Additional on-site treatments may be necessary to comply with environmental regulations.

Exhaust from the fermentation process is typically treated for a variety of reasons such as odor control, environmental compliance, contamination prevention, and preventing the uncontrolled release of the production strain. Vent treatment methods include but are not limited to: Chemical Scrubbing, Filtration, and Thermal Treatment.

Recommended best practices

- Maintain good housekeeping in waste collection and treatment areas
- Avoid standing water as much as possible in processing area and premises
    - This includes avoidance of open drainage
- Avoid aerosol development as well as dust
- Separate areas/ equipment dedicated for the containment of waste material must be provided and these areas/ equipment must be properly maintained
- Use closed containers, closed systems or separate rooms to segregate waste from the rest of the process
- Any container, room etc. used for waste collection of treatment must be marked accordingly
- Consider devitalization/deactivation of your biological active waste prior to disposal
- Waste treatment processes may utilize public systems for disposal where permitted
- Obey country specific and local requirements for waste disposal.

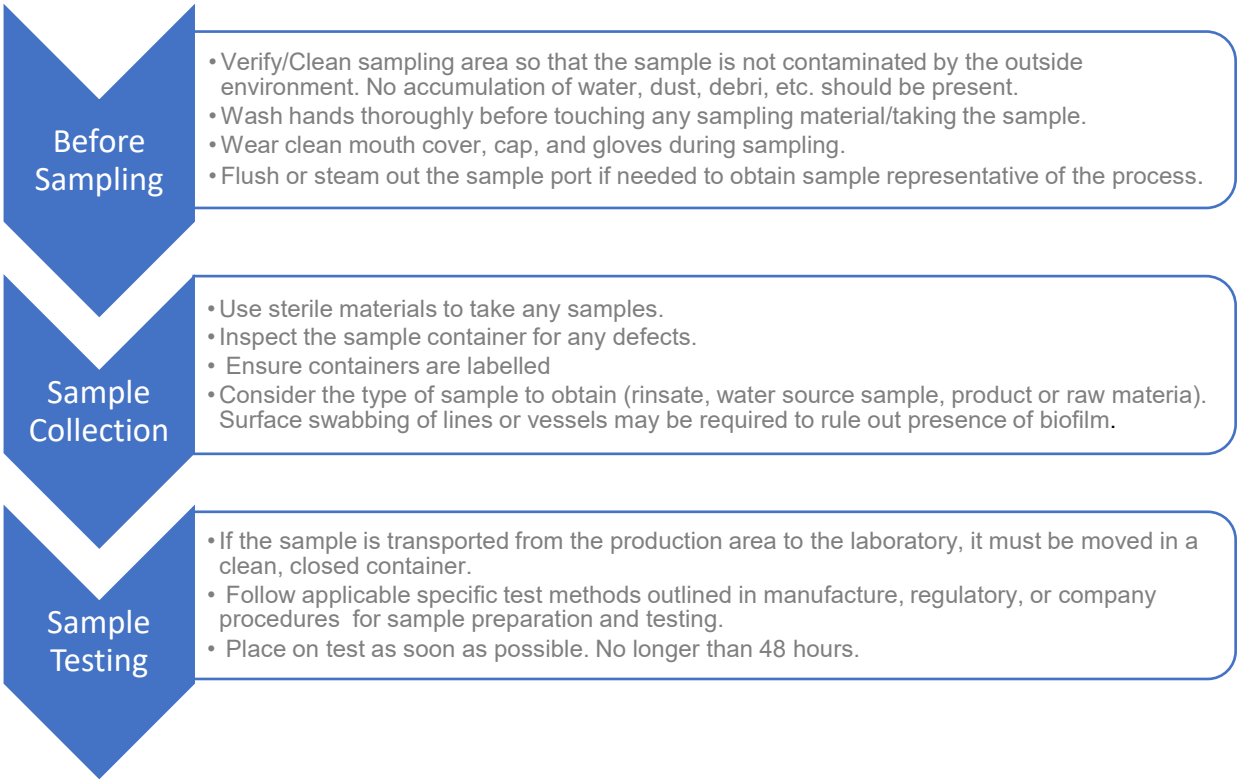
10 Contamination Testing

A key requirement in testing of any agricultural biologic is to ensure there is a validated method available to identify and quantify the desired micro-organism and to discriminate the desired from the undesired micro-organisms.

10.1 Sampling Practices

It is important to ensure that the samples taken in the different stages of the processes are carried out properly, identified through labelling, and recorded to avoid possible rework, mix-up, false positive or negative results. Samples should be taken in a manner that protects both the sample as well as the process from inadvertent contamination. The guidelines presented in this sub-section are not just limited to samples collected for contamination testing but to process sampling in general where contamination of any sample may have undesired effects on the test results.

Some recommendations are:



Sampling is an important operation in which only discrete amounts of a batch of a material is collected with the purpose of evaluating the quality of the whole batch. Valid conclusions on the quality of an entire batch can only be based on tests which are carried out on representative samples. Correct sampling is thus an essential part of a system of quality assurance.

The sampling plan developed as well as specification of the sample storage requirements should be appropriately justified and based on the requirements of process monitoring and in-process and product variability characterization as well as requirements for regulatory, and marketing.

The sampling plan and associated sampling procedures shall take into consideration the following:

- Form of the material - solid, liquid
- Time and point of process from which the sample is taken
- Type of container being sampled - cans, drums, bags, bulk facilities, etc.
- Frequency and composition
- Sample size, the amount to be collected
- Sample preparation, including sub-sampling, while ensuring homogeneity
- Sample transportation and storage as needed.

Materials and equipment used for sampling and short-term sample storage shall consider the following:

- All material used for sampling should be clean, dry, sterile, and placed in clean areas to avoid contamination.

Broken, or materials not approved for reuse should be removed.

Samples should be prepared and stored appropriately according to specific test methods, manufacturer, or regulatory guidelines.

Sample container should be identified with the material, batch, or lot number, and/or sample number, production date and hazard classification.

Long-term storage of samples and retains will also be required and will likely result in the need for a specialized storage area with area specific requirements and procedures. For the design of the area and within the procedures the following aspects should be considered:

- Define retention period of the finished good samples
- The storeroom conditions must be capable of meeting the ingredients and materials storage requirements to provide a standardized and safe continuous storage of the material
- The sample storeroom must have sufficient capacity
- The sample storage area should provide:
    - Temperature control
    - Inventory management
    - Separation as required
    - Security aspects / Access authorization
    - Emergency plan e.g. spill program (where it applies).
    - Monitoring or recording of the storage room environment

10.2 Contamination Testing Requirements Including Human Pathogen Screening

Based on the potential risk to introduce unwanted and possibly hazardous micro-organisms, country regulatory bodies require screening for human pathogen in addition to general contamination tesing. Industry best standards have also been established by, for example, the OECD. European member states require testing following the OECD list, while other countries, like the US and Canada, have a shorter list.

As a result, in order to develop the appropriate contamination testing plan, the country specific regulatory requirements must be known and followed.

After tests that meet the regulatory requirement have been minimally included in the plan, additional tests or stricter limits based on product safety may be implemented.

Below is a process flow chart to help establish a testing plan to monitor for microbial contamination and human pathogens in the final product.

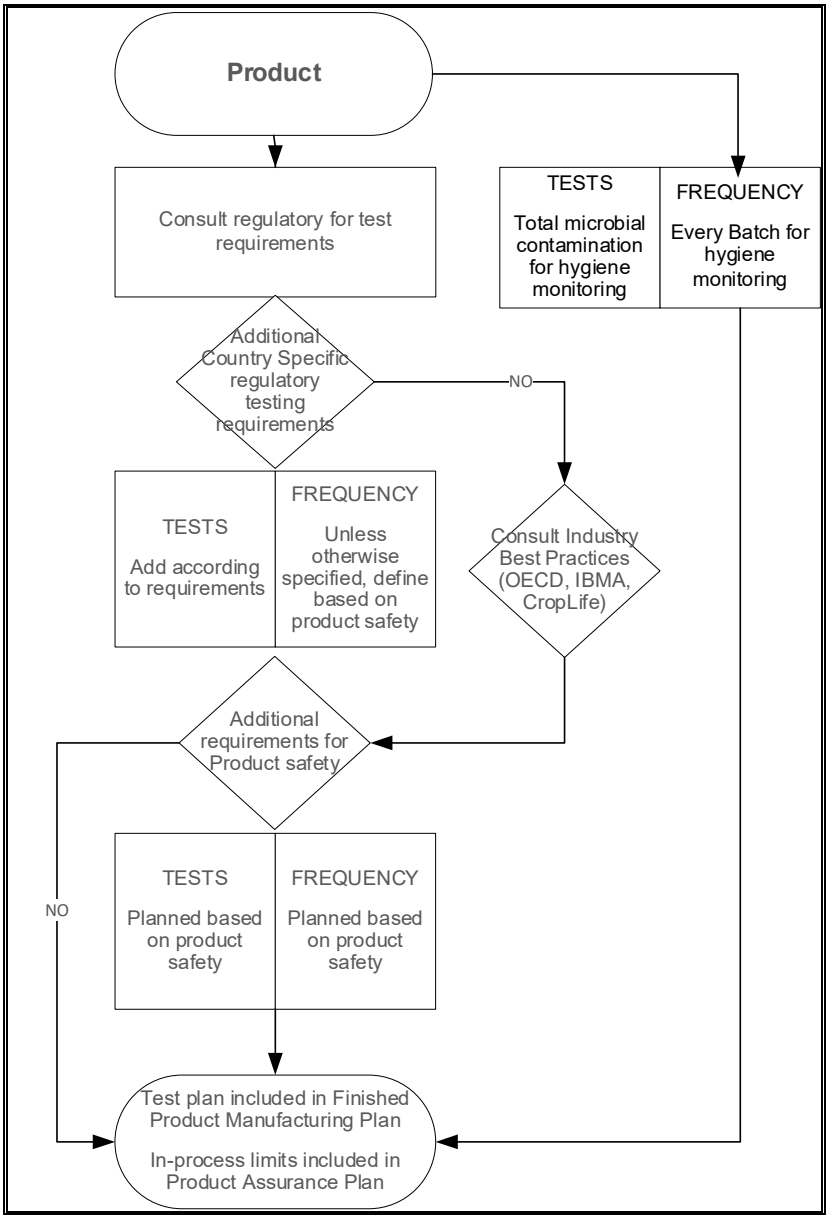


Figure 3: Process flow to establish test plan for pathogen testing

10.2.1 Contamination Limits and Rational for Testing in Agricultural Biologicals

Below is an example of human pathogen limits taken from guidance document "OECD Issue paper on Microbial Contaminant Limits for Microbial pest Control Products No. 65".

Microbial Activity Indicator	OECD Recommended Limit CFU (Colony Forming Unit)	OECD Rationale for Testing
Aerobic bacteria	<10E5 CFU/ g or CFU/ml	Indicator for aerobic bacterial contamination; optional if microbial pest control agent (MPCA) is an aerobic bacterium.
Anaerobic spore-formers	<10E5 CFU/ g or CFU/ml	Indicator for hygiene failures during manufacturing; optional if other indicator strains (i.e., E. coli and S. aureus) are screened during manufacturing or if MPCA is a microaerophile or spore former.

Yeast and Mold	<1000 CFU/ g or CFU/ml	Indicator for fungal and mold contamination and potential mycotoxin presence.
Pathogenic Indicator	**OECD Recommended Limit**	**Rationale**
Salmonella spp.	absence in 25 (g or ml)	Widely used indicator for hygiene and standard methods available.
Listeria monocytogenes	absence in 25 (g or ml)	Can multiply under refrigerated conditions; may be optional if other indicators are acceptably low.
Vibrio spp.	absence in 25 (g or ml)	Additional requirement recommended if high potential for contamination or Vibrio naturally occur at manufacturing site.
Shigella spp.	absence in 25 (g or ml)	Additional requirement recommended if high potential for contamination or Shigella naturally occur at manufacturing site.
Escherichia coli **Or** **Thermotolerant Coliforms**	absence in 1 (g or ml) < 10 CFU/g or ml	Indicator of fecal contamination, can multiply/survive on plants, and in soil and water.
Staphylococcus aureus	absence in 1 (g or ml)	Indicator of contamination due to improper handling (hygiene)
Pseudomonas aeruginosa	monitoring (to be evaluated if positive)	Indicator of environmental contamination; recommended if screening for other hygiene indicators suggest presence of pseudomonas.

Table 5: OECD Microbial Contamination Limits

Pathogen limits of EU Fertilizer act (2019-1009) for microbial based Bio Stimulants

Microbial Activity Indicator	Limit	Pathogenic Indicator	Limit
Anaerobic plate count	<10E5 CFU/ g or CFU/ml	Escherichia coli	Absent in 1g or 1ml
Yeast and Mold	<1000 CFU/ g or CFU/ml	Staphylococcus aureus	Absent in 25g or 25ml
		Salmonella spp.	Absent in 25g or 25ml
		Shigella spp.	Absent in 25g or 25ml
		Listeria spp.	Absent in 25g or 25ml
		Vibrio spp.	Absent in 25g or 25ml
		Enterococcaceae	Absent in 25g or 25ml

Table 6: EU Fertilizer Microbial Contamination Limits

10.3 General Contamination Test Methods

Different types of microbial test methods are available to support manufacturing processes based on the need for information. The following methods may be used for cleaning verification, in-process or envrionmental monitoring.

Detection of the presence or absence of microorganisms:

Presence / absence tests are designed to detect the presence of any organism in a sample or to detect a specific microbial species. Simple and rapid methods (in the case of the last three ones mentioned) include the dipstick, ATP (BacTrac®, 3M®), CO2 sensor (Certa Blue®, BioLumix®), which can give semi-quantitative results.

Enumeration of microorganisms:

Enumeration tests determine how many microorganisms are present in a sample.

The ability to enumerate microorganisms is influenced by many factors, including condition and duration of incubation, incubation temperature and whether the organism is stressed or shocked. There are two overall methods to choose from: direct Agar plating (e.g. Standard Plate Count) or membrane filtration followed by transfer to an Agar plate.

Identification of microorganisms:

Identifying the microorganisms present in the test sample can help to determine the source of the contamination :

- Pseudomonas species may indicate a contaminated water source
- Staphylococcus species point at a personal hygiene issue
- Bacillus species are an indicator of inadequate sterilisation
- Yeasts and molds can point to facility issues, e.g. air contamination.
-

Test Method	Prescription	Advantage	Disadvantage
Dip Slide	Dip slides contain agar media on a plastic support. To be used to detect MC in liquids	Easy to use, inexpensive, test for viable microbes	Incubation time is needed, use for liquids only
Petri Film	To be used to detect MC on surfaces or in liquids	Easy to use, no autoclave needed	Difficult identification of colonies
ATP Testing	To be used to detect MC in liquids and surfaces	Immediately results, easy to use	Does not differentiate between viable and non-viable organisms, cannot be used in final product or thickeners
Electric Impedance Analyzer (e.g. Bac Trac)	Bacteria or yeast/ mold grow in nutrition medium Impedance signal change over time is measured	Very fast detection of MC, large number of samples can be handled, detection of viable organism	Difficult to use, expensive equipment, needs experienced staff, intensive calibration
Standard Plate count	To be used to detect MC on surfaces or in liquids	Easy to use, inexpensive, test for viable microbes in liquids, suitable for dry or wet swab testing	Incubation time is needed
BioLumix (CO_2 sensor)	Ready-to-use sterile vials available with different types of media Depending on the medium chosen, method can be used to detect total aerobic bacteria, specific bacterial species, yeasts, or molds		Incubation time is needed

Table 7: Comparison of test methods

11 Microbial Environmental Monitoring Program

Baseline microbial monitoring of the process stream, sometimes called microbial mapping should be performed to understand the initial microbial load of a process system or unit. The data from this process should be used to set up an ongoing routine process monitoring system and become part of a continuous improvement plan.

After defining and mapping baseline testing areas in the facility, frequency of testing needs to be decided. Initial frequency of testing should be higher and be representative of typical working conditions to collect sufficient data to establish a baseline trend. Data may be variable due to limited quantitative accuracy, and in such case, increasing the number of sample replicates per timepoint can be considered to reduce the variability.

Also, consideration should be given to taking measurements in both active and inactive periods to compare the impact of a specific activity. Once an acceptable baseline has been established frequency of testing may be reduced, thus allowing for regular or periodic monitoring.

Air:

Microbial contaminats from the air can be tested either actively or passively.

Active monitoring makes use of an air sampler that physically draws a known volume of air through or over a particle collection device which can be a liquid or a solid culture media or a nitrocellulose membrane.

Passive monitoring includes using “Settle plates” (standard petri dishes containing culture media) which are strategically placed and exposed to the air for a prescribed time to collect bacterial and fungal particles which lands on the plates and are then incubated for growth. Results are expressed in CFU/plate/time.

Passive sampling is not as sensitive as active monitoring however still provides a valid risk assessment as it measures the airborne population which falls onto a critical surface during a certain timepoint.

Water:

Cultural methods are still commonly used for water testing where the preferable method involves using membrane filtration due to the large sample size and low limit of detection. With these methods, performing the test on the sample as soon as possible after collection is important.

Testing or post-cleaning rinse water, using the same methods as process water, may provide a useful indication of cleaning effectiveness and microorganism buildup over time. It may also be used for continued monitoring to determine completion of cleaning before proceeding to next production campaigns or batches.

Equipment and Surfaces:

Surfaces may be monitored for the presence of contaminants by making use of sterile swabs. Many different types of swabs are available including wet or dry, small bud or large sponge types. Wet swabs are easier for swabing dry surfaces and, for convenience, the liquid may be poured onto agar or Petrifilm. Large sponge swabs can be used to swab larger surface areas. After swabbing the area it is important to transfer the liquid as soon as possible onto an agar medium.

Dip slides are another easy and cost effect method used to detect contaminants on a surface.

ATP swabs can also be used as a fast indicator that the surface is not adequately cleaned. Levels of Adenosine Triphosphate (ATP) molecules are measured in a handheld device and provide a fast method to measure contamination before and after cleaning a surface.

12 Cleaning, Disinfection, and Sterilization of Equipment

Biotechnical production plants and their equipment have to be adequately and routinely cleaned and disinfected. This is essential to minimize the risk of product contamination. Detailed procedures must be developed for all surfaces that come in direct contact with the product(s) (e.g. the inside of vessels, pipes, utensils, etc.).

Similarly, appropriate cleaning procedures are required for all “non-product contact” surfaces. This should consider general hygiene of the external surfaces of equipment, overhead structures, shields, floors, walls, ceilings, lamps and other lighting devices, refrigeration units, heating, ventilation and air conditioning systems, etc.

Achieving the required level of cleaning effectiveness and disinfection is based on choosing on the right cleaning and disinfection or santization chemical agents, using them at the right concentration for the right amount of contact time at the right pH and temperature condition. Additionally, re-circulation or dynamic movement of the chemical cleaner or sanitizer through the equipment is more effective than static application of the cleaner or sanitizer.

Cleaning and sanitization programs should be periodically reviewed and verified on a product by product basis, over the entire manufacturing process and for the life of the manufacturing plant.

Biofilms are a particular challenging subtype of microbial contamination. Therefore, the cleaning regiment developed needs to consider and address this type of contamination.

Biofilms are most often identified by the following:

- Persistant positive contamination results with similar or same organism identified
- Visual appearance of a thick mucoid (slimy) coehesive layer within a piece of equipment or system
- Biofilm-producing organisms may appear different when cultured in lab conditions
- During organism ID and physical testing it can be determined if an organism is a biofilm producer.

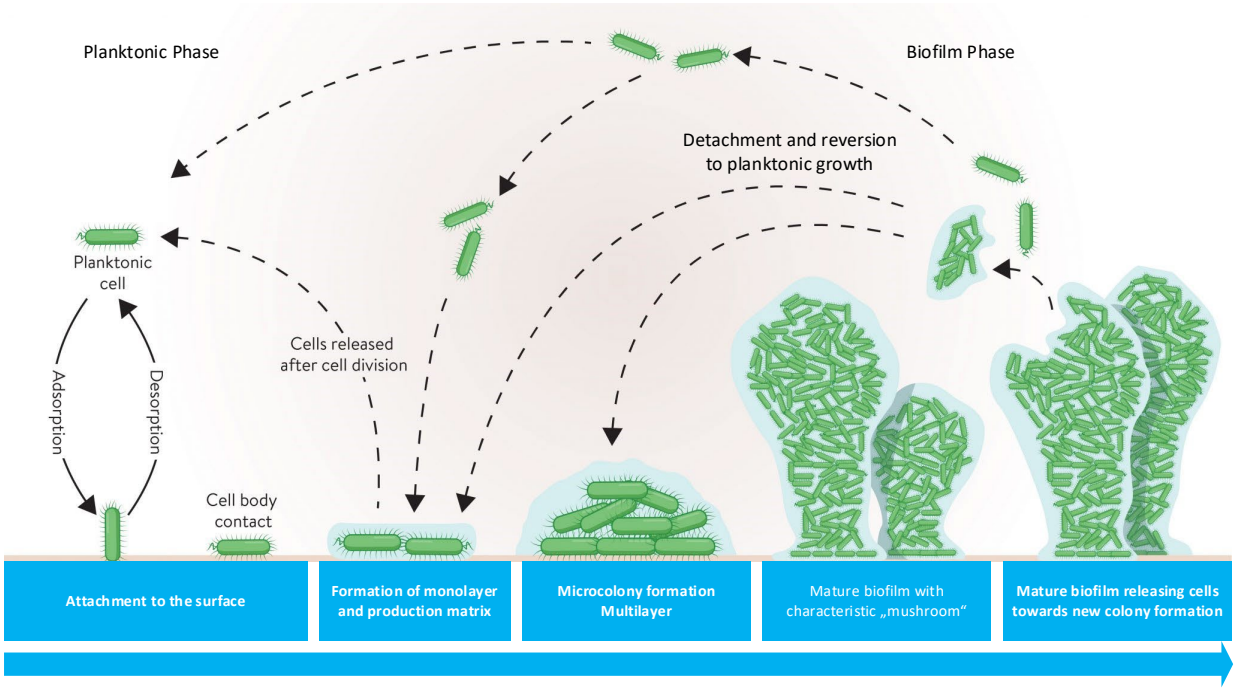


Figure 4: Phases of Biofilm development

12.1 Cleaning Procedures

To prevent microbial contamination consistently, a high level of control of potentially contaminating microorganisms is mandatory not only in the actual manufacturing equipment, but also in the entire manufacturing area.

Besides the products manufactured, the plant and equipment design, severity of the microbial risk, available cleaning / disinfectant products, waste disposal limitations and legislation have to be taken into consideration.

Therefore, it is necessary that each manufacturing unit be considered individually so that specific cleaning strategies can be implemented.

The cleaning, disinfection and sterilization procedures have distinct process steps. However, these process steps are firmly interrelated, and the result will only be acceptable when all procedures have been carried out correctly in the right order.

Successful, consistent management of microbial contamination can only be achieved if all locations in the equipment and in the plant in which microbial growth can occur, are accessible for cleaning and disinfection. Therefore, one or all of the following cleaning methods may have to be used:

- **Manual cleaning**: complete disassemble for manual cleaning and inspection
- **Clean-in-place (CIP): equipment systems designed** for automatic cleaning and disinfection without disassembly
- **Clean-out-of-place (COP)**: partially disassemble equipment and clean in specialized areas
- **Steam-in Place (SIP)**: systems designed for automatic disinfection with steam without disassembly
- **Autoclaving (COP)**: Autoclaving using elevated temperature to sterilize mobile equipment or parts.

There are a number of additional important rules that must be implemented at all stages in the cleaning and disinfection procedures of equipment and plants:

- Determine the presence of Biofilm formation within a system
- Before starting the cleaning, clear the whole area, i.e. remove any product, raw materials, bins, labels, tools and all other portable equipment (which will have to be cleaned separately)
- Include a status label of the equipment or process unit to communicate that cleaning is required and has not yet been completed. This can prevent confusion of mistakingly using equipment that has not been cleaned in the next process
- All cleaning and sanitization steps must always follow proven and established procedures following the same sequence as the manufacturing steps, i.e. starting with the staging of raw materials down the production line to the point where the end product is collected
- Sanitization equipment assigned to specific critical steps should be dedicated
- Dismantle, if necessary, the equipment in order to expose all the surfaces for cleaning
- Inspect gaskets, seals, filters etc. determine appropriate maintenance schedule for replacement
- Ensure the lines can be drained at the lowest point to prevent trapping liquid (which could harbour microbial growth).

- Always check that all the cleaning solutions have been completely drained before rinsing.
-

Figure 5: Cleaning sequence

12.2 Chemical, Mechanical Cleaning and Rinsing

Pre-rinse is performed to remove any loose residue materials left in the manufacturing equipment.

After the pre-rinse, chemical and mechanical cleaning can be performed and involves the use of chemical cleaning agents and mechanical forces to remove all remaining residual material. This may involve scrubbing parts / surfaces with a brush or the use of high-pressure water blasting or high velocity flow while using an apporpriate cleaning agent. The specific cleaning chemical and mechanical action used for a piece of equipment or system depends on the nature of the residual material, the type of equiment and chemical compatability between the cleaner and the equiment and its components.

General classes of cleaning chemicals include:

- Caustics
- Acids
- Solvents
- Detergents

Manual cleaning by dissasembly and scrubbing is typically implemented for smaller parts and surfaces. It is recommended to move and clean small equipment, tools, utensils (e.g. brushes), parts and fittings in a special dedicated area. Utensils must be cleaned immediately after use, dried, properly labeled and stored under sanitary conditions. It is recommended to have dedicated carts and/or racks in place for temporary storage of the cleaned parts before reassembly. It is recommended to use labels to show status of equipment clean or dirty. Cleaning agents used to perform manual or COP operations must not pose significant safety hazard to workers.

A high-pressure application of cleaner is generally used for larger parts in the manufacturing plant such as vessels / tanks, agitators, large piping, and fixed connected charging systems. Mechanical application of a cleaning agent and/or rinse techniques may be performed by spray balls. These can be installed in process vessels and are routinely used to distribute cleaning solution and ensure the entire surface area coverage. The mechanical energy and chemicals involved in this type of operation may pose an elevated hazard to employees so this often necessitates that the equiment or system is entirely closed during cleaning. Additionally, the high-pressure may create aerosols which could spread the microbial contamination if the system was not closed.

Pipeline cleaning pigs or scrapers can be used to mechanically remove biofilms, solids buildup or cleaning chemicals. When pigs or scrapers are not avaialble or practical, high velocity circulation of the cleaning solution can be used. This approach requires that the piping is completely filled and circulation is performed at a prescribed rate and temperature for a prescribed time.

Following chemical and mechanical cleaning, all parts, equiment and systems must be thoroughly rinsed and drained to completely remove the chemical cleaner. Having a method to verify complete removal of the chemical following rinsing is highly desirable. Verification may involve use of pH or conductivity measurments or a chemical specific test method.

12.3 Water Source Used for Equipment Cleaning

When defining liquid phase cleaning/sanitization processes, consideration should be given whether the quality of the water used needs to be the same as the process water.

Neutral water pH levels are considered suitable for most detergents and disinfectants. Highly alkaline or highly acidic water may require additional buffering agents. Hard water needs to be avoided as it leads to precipitation of insoluble salts which form scales and reduce the effectiveness of detergents and heat transfer devices and can promote biofilm formation.Reduction in the formation of scales can be achieved by addition of chelating and sequestering agents, which bind calcium and magnesium.

12.4 Chemical Disinfectants / Sanitizers

Chemical cleaning may not be sufficient to eliminate microorganisms from a process. Therefore, a disinfectant / sanitizer may be needed. Alkalis, acids and solvents can serve as disinfectants.

Disinfectant Type	Effective Against	Cautions	General contact time
Bleach; Hypochlorite (Household bleach 5% diluted 1:10 for 0.5% or 1:100 for 0.05 % solutions)	Most bacteria (including spore forming bacteria), fungus, mold, and viruses. Broad spectrum; unaffected by water hardness, quick acting. Low cost.	Can be corrosive to metal, skin irritant, toxic to aquatic life. Higher concentration may be required especially for high bioburden.	10-30 min
Alcohols (70% Isopropyl Alcohol or Ethanol)	Bacteriocidal, fungicidal and virucidal. Ineffective against spores or protein rich materials.	Flammable, evaporative, can damage materials after prolonged use	10-60 sec
Hydrogen Peroxide (3-5%)	Most bacteria, spores, fungi, viruses	Generally considered safe with low environmental footprint	15-60 min
Peracetic Acid	Most Bacteria, fungi, viruses, spores. Water soluble.	Rapid acting, flammable, and explosive at high temperatures.	10-15 min
Quaternary Ammonium	Most Gram positive and negative bacteria, enveloped viruses, and some fungi.	Not effective against Pseudomonas species or Staphylococcus aureus, or spore forming bacteria. Substantial interference when there is organic matter in matrix.	10-15 min
Sodium Hydroxide 0.4-4%	Most yeast, fungi, bacteria but not as effective on spore forming bacteria.	Can be very corrosive	1-2 hours

Table 8: Types of Disinfectant agents

Irrespective of the choice of cleaner or disinfectant, it must be ensured that the required concentration is used, and the prescribed contact time and temperature are implemented. A good practice is to rotate between different chemical disinfectants to sustain a low level of background environmetal contamination.

Testing final rinse water for evidence of chemical residue, e.g; pH or chemical test kit, is critical to batch manufacturing startup.

12.5 Sterilization of Equipment and Processes by Steam

Sterilization is performed when bioburden reduction beyond sanitization is required. Sterilization can be required for equipment and processes where bioload reduction is critical to ensure that the final product is not contaminated.

The sterilization of inputs (e.g. fermentation media, water, air) is discussed in chapter 9.8.

The minimum sterilization condition is typically considered 121°C for 12 min after the system temperature has been reached. However, the sterilization time and temperature conditions are dependent on the system design and to meet the specific process needs.

There are numerous considerations for usage of steam.

An important consideration for thermal sterilization is that all parts of the line are heated up to the required temperature and incubation time. A thorough review of equipment and piping configuations must be performed to ensure there are no dead-legs.

Temperature mapping is a good way to validate that sterilization temperatures are met within the entire equipment and piping system. During the cool down phase, it is important to use sterilized process air/gas to avoid vacuum and to ensure positive pressure on the system. Equipment must be specifically designed to handle both sterilization temperature and pressure operating conditions including full vacuum.

Key Sterilization Reference:

Steam Sterilization Principles. Marcel Dion and Wayne Parker. ISPE Pharmaceutical Engineering December 2013.

12.6 Disinfection by UV Light

UV light is used to minimize bioburden on surfaces in clean rooms and inside laminar flow. Intensity, spectrum and exposure time need to be monitored to ensure effectiveness. Ensure that surfaces are free of obstruction, and there should be no debris or shadows.

12.7 Drying

Microorganisms need water to grow. Between campaigns and after cleaning and sanitizing the surfaces of equipment should be properly drained and dried. Flexible hoses should be removed and hung in an inverted U to ensure draining and drying.

13 Glossary

Terminology and perception differ greatly between biotechnology disciplines. The glossary summarizes the most reasonable definitions from the author point of view.

Name	Abbrev.	Description / definition
Aseptic		Free from contamination caused by unwanted bacteria, viruses, or other microorganisms.
Agricultural Biologicals		A biological is an agriculture solution that is derived from naturally occurring or living materials.
Biochemical oxygen demand	**BOD**	The amount of dissolved oxygen required to meet the metabolic needs of aerobic microorganisms in water rich in organic matter, such as sewage.
Biocontamination		Contamination of materials, devices, individuals, surfaces, liquids, gases, or air with viable particles (e.g., microorganisms, spores or phages).
Biofilm		A group of microbial cells adhering to a surface.
Bioprocess		A technique that produces a biologic material for commercial use. Often used specifically to produce chemicals or fuel by biologic processes, such as microbial fermentation or degradation.
Biosafety		Development and implementation of administrative policies, work practices, facility design, and safety equipment to prevent transmission of biologic agents to workers, other persons, and the environment.
Biosafety level	**BSL**	A biosafety level is a level of the biocontainment precautions required to isolate dangerous biologic agents in an enclosed laboratory facility.
Bio stimulant (Plant)		A substance, microorganism, or mixture thereof, that, when applied to seeds, plants, the rhizosphere, soil, or other growth media, act to support a plants natural process independently of the bio stimulant's nutrient content, including by improving nutrient availability, uptake or use efficiency, tolerance to abiotic stress, and consequent growth, development, quality, or yield.
Biotechnology, biotechnological		The use of microorganisms, such as bacteria or yeasts, or biologic substances, such as enzymes, to create specific intermediates and products.
Broth (fermentation)		Fermentation media including cells, products (intermediates) and waste material.
Cell clone		Cell clones are genetically identical cells.
Chemical Oxygen Demand	**COD**	Oxygen demand is determined by measuring the amount of oxidant consumed using titrimetric or photometric methods.
Clean area		Clean area is an area with defined environmental control of particulate and microbial contamination, constructed and used in such a way as to reduce the introduction, generation, and retention of contaminants within the area.
Clean room		Clean room is a room in which the concentration of airborne particles is controlled, and which is constructed and used in a manner to minimize the introduction, generation, and retention of particles inside the room and in which other relevant parameters, e.g. temperature, humidity, and air pressure, are controlled as necessary.

Name	Abbrev.	Description / definition
Cleaning		Considered to be a primary step of physically or mechanically removing dirt/ debris or residue from a surface.
Cleaning-In-Place	**CIP**	Automated or manual cleaning system of a process line and/or individual equipment in a closed circuit without dismantling.
Cleaning-Out-of-Place	**COP**	Equipment is partially disassembled and cleaned in specialized areas or containments.
Closed processing or closed systems		Closed processing or closed systems are process steps (or systems) which separates the process from the environment. Material (contaminations) cannot enter, nor can product leave the system and product is not exposed to the immediate room environment. [Odum2010]
Colony-Forming Unit	**CFU**	In microbiology, it is a unit which estimates the number of microbial cells in each sample.
Contaminants		Any biologic or chemical agent, foreign matter or other substances not intentionally added to the product that may compromise suitability or safety.
Critical control point	**CCP**	A point where failure of a standard operation procedure (SOP) could cause harm to customers and to the business.
Disinfection		The process of using a disinfectant to destroy, inactivate, or significantly reduce the concentration of unwanted microorganism to ensure a microbial acceptable standard of hygiene is achieved.
Failure Mode and Effects Analysis	**FMEA**	An analytical tool that uses a disciplined technique to assess and prevent failure modes within product and process systems. An organism whose genetic material has been modified or altered by genetic engineering methods.
Good manufacturing practice	**GMP**	Good manufacturing practice (GMP) is that part of quality assurance which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the marketing authorization.
Hazard		A biologic, chemical or physical component or condition with the potential to cause an adverse health effect.
Hazard analysis and critical control points	**HACCP**	System which identifies, evaluates and controls hazards which are significant for food safety
Inoculation		The introduction of a microorganism to a growth medium (or into a host organism)
Master Cell Bank	**MCB**	The MCB is a collection of aliquots of a targeted pool of cells which generally has been prepared from the selected cell clone. The MCB is used to derive all working cell banks.
Media simulation		Process run with pure media without bacterial culture.

Name	Abbrev.	Description / definition
Medium (culture/ growth/ fermentation)		Growth medium or culture medium is a liquid or gel designed to support the growth of microorganisms by e.g., supplying nutrients.
Microorganisms	**MO**	Any single celled organism, such as a bacterium, protozoan, or virus, of microscopic size.
Monitoring		The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a parameter is under control.
Non-product contact surfaces		All exposed surfaces other than those coming in direct contact - or potential - contact with product.
OECD		Organization for Economic Co-Operation and Development
Pathogen		Any virus, microorganism, or other substance causing health disease or condition.
PCMCCPP	**PCMCCPP**	Prevention and Control of Microbial Contamination in Crop Protection Products
Product contact surfaces		All equipment surfaces that intentionally or unintentionally (e.g., due splashing) encounter the product, or from which product or condensate may drain, drop or be drawn into the main product or container.
Quality Assurance / Quality Management	**QA/QM**	This can be defined as all the activities and functions concerned with the attainment of quality in a company. In a total system, this would include the technical, managerial, and environmental aspects.
Quality Control	**QC**	Can be defined as the operational techniques and activities that are used to fulfil quality requirements
Risk		A measure of the potential loss of a specific biologic agent of concern, based on the probability of occurrence of an adversary event, effectiveness of protection, and consequence of loss
Sanitization		The reduction of the number of microorganisms in the equipment or environment to a level that does not compromise product safety.
Separation		Separation is the purposeful creation of a barrier between incompatible substances so they can never come together. Separation by physical, air, and personnel of rooms or buildings, where material or equipment cannot be shared due to high risk from contamination.
Scale-up		steps involved in transferring a manufacturing process or operation from laboratory scale to the level of a commercial product

Name	Abbrev.	Description / definition
Seed fermenter		Seed fermentation allows the cells from the inoculum to reproduce and adapt to the environment and nutrients that they will encounter later in the production.
Soil		Any remaining, undesirable material in the equipment or process environment.
Sparger		A sparger in a fermenter disperses air into a broth
Standard Operation Procedures	**SOP**	Detailed, written instructions to achieve uniformity of the performance of a specific function.
Sterilization		A process affected by chemicals, heat, or other physical means (e.g., irradiation), aimed to remove or kill all forms of microorganisms.
Sterile product		Free of all microbe organism not desired
Steam-In-Place	**SIP**	Steam used to sterilize equipment without dismantling.
Strain (bacteria)		A genetic variant within a species
Submerged fermentation		A method for growing cultures of bacteria in which microorganisms are incubated in a liquid medium subjected to continuous, vigorous agitation.
Substrate		A surface on which an organism grows on or is attached to, or the substance that is acted upon by an enzyme or fermenter
Surface treatment		A process by which chemical or mechanical properties of existing surfaces are altered.
Thermal disinfection		Thermal disinfection involves the use of hot water or steam for a specified temperature and contact time.
Validation		Confirmation/ testing that a process or system meets its pre-determined specifications and quality attributes.
Working Cell Bank	**WCB**	The working cell bank (WCB) is a culture of cells derived from the master cell bank and intended for use in the preparation of producing cell cultures within an ongoing manufacturing setting.