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Monoclonal antibodies manufacture for cancer therapy.

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In life there is nothing to fear, only things to understand.

Marie Curie

Dedicat a la meva mare, per ser el principal motiu d'interès en aquesta tesi.

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SUMMARY

Monoclonal antibodies (mAbs) specifically target a particular antigen. The increase in cases of diseases such as cancer has been the main cause of increased research within the field of therapeutic mAbs which have steadily grown to become a dominant product in the biopharmaceutical market.

The objective is to develop a process for the manufacture of a monoclonal antibody, through a literature search, Bevacizumab has been selected. In clinical practice it is used for the treatment of multiple cancers and is the only one capable of slowing the growth of advanced stage epithelial ovarian cancers.

Regulatory compliance is studied with reference to the main aspects of safety and hygiene, based on state and international public regulations and their subsequent application to the plant.

The synthesis of the preparation process is the same for all mAbs, consisting of upstream cultivation stages, with four reactors in series of increasing size for fermentation and subsequent centrifugation, and downstream purification stages, with first filtration, followed by the corresponding chromatography's and finally ultrafiltration.

Basic design of the manufacturing plant was carried out using Aspen Batch Process Developer program. Operations defined in synthesizing chapter were introduced in program for a batch load of about 50 kg, and equipment from local suppliers was selected. Aspen scheduling shows that upstream process is time limiting, and that 20 batches/year can be carried out resulting in a production of about 1000 kg mAb/year.

Keywords: Monoclonal antibodies, cancer therapy, Good manufacturing practice, process synthesis, basic design.

RESUMEN

Los anticuerpos monoclonales (mAbs) tienen como objetivo específico un antígeno en particular. El aumento de casos de enfermedades como el cáncer ha sido la principal causa de la mayor investigación dentro del campo de los mAbs terapéuticos que han crecido de manera constante hasta convertirse en un producto dominante en el mercado biofarmacéutico.

El objetivo es desarrollar un proceso para la fabricación de un anticuerpo monoclonal, mediante una búsqueda bibliográfica, se ha seleccionado el Bevacizumab. En la práctica clínica, se utiliza para el tratamiento de cánceres múltiples y es el único capaz de disminuir el crecimiento de los cánceres ováricos epiteliales en etapa avanzada.

Se estudia el cumplimiento de las normativas haciendo referencia a los aspectos principales sobre seguridad e higiene, basados en la normativa pública estatal e internacional y su posterior aplicación a la planta.

La síntesis del proceso de preparación es igual para todos los mAbs, consiste en etapas upstream de cultivo, con cuatro reactores en serie de tamaño creciente para la fermentación y una posterior centrifugación, y, en etapas downstream de purificación, con una primera filtración, seguida de las correspondientes cromatografías y finalmente una ultrafiltración.

El diseño básico de la planta de fabricación del Bevacizumab se realizó con el programa Aspen Batch Process Developer. Las operaciones definidas en el capítulo de síntesis se introdujeron en el programa para una carga de lotes de unos 50 kg, y se seleccionaron equipos de proveedores locales. La programación de Aspen muestra que el proceso upstream está limitado en el tiempo, y que pueden llevarse a cabo 20 lotes/año, lo que resulta en una producción de unos 1000 kg de mAb/año.

Palabras clave: Anticuerpos monoclonales, terapia contra el cáncer, buenas prácticas de fabricación, síntesis del proceso, diseño básico.

1. INTRODUCTION

The immune system is the main defence mechanism against substances that have managed to penetrate. An antigen is a substance capable of causing such a reaction by the immune system.

Antibodies are found in the globulin fraction of proteins circulating in the blood and are known as immunoglobulins. They are grouped into five main classes according to their physical properties, such as molecular weight, and are designated by a letter associated with each class.

La fuerza con la que un anticuerpo se adhiere a un antígeno se denomina afinidad. En función del uso para el que se requiera el anticuerpo, es posible que la fuerte unión del anticuerpo al antígeno sea, en distintas circunstancias, algo bueno o malo.

Nuestro sistema inmunitario arremete contra todo tipo de sustancias extrañas produciendo una gran cantidad de anticuerpos. Un anticuerpo es una proteína que circula por todo el cuerpo hasta que localiza y se adhiere a una proteína específica llamada antígeno (Ruiz et al., 2007).

Antibodies can be designed to specifically target a particular antigen, such as can be found on cancer cells, and then many replicates of that antibody can be made in the laboratory. They are called monoclonal antibodies (mAbs or MoAbs) and the particularity that makes them unique is that all molecules in any preparation are identical, their reaction with a defined antigen must always be the same. It is precisely this constancy in their preparation and effect that makes them so useful and useful (American Cancer Society, 2019; Sikora and Smedley, 1986).

Since their discovery, mAbs have been a key research tool in the age of biotechnology, opening up many new areas of biological and clinical research. They have led to the evaluation of previously unmeasurable substances, the determination of new cell populations and the discovery of new differentiation pathways. They are destined to revolutionise all areas of laboratory medicine, as well as providing exciting therapeutic opportunities for patients with cancer, infectious diseases and autoimmune disorders (Sikora and Smedley, 1986).

MAbs are artificial proteins developed to serve as human antibodies that can repair, enhance or mimic the immune system's attack (American Cancer Society, 2019). In theory, they are characterised by a unique primary amino acid structure. However, in reality, a single dose of a mAb product involves a multitude of variants inherent to the biotechnological process used to manufacture pharmaceuticals (Carrara et al., 2020).

In recent years, they have become increasingly important in the fight against various diseases (Carrara et al., 2020). They have become the reference treatment modality in several therapeutic areas, such as oncology, haematology and immunology (Lu et al., 2020). They have steadily grown to be the dominant product class within the biopharmaceutical market, thanks to their high specificity, a consequence of their complex glycoprotein structure (Carrara et al., 2020).

Some mAbs used for cancer treatment are known as targeted therapy because they have a specific target on a cancer cell they encounter, attach to it and attack it. But others behave like immunotherapy because they make the immune system react better so that the body targets and attacks cancer cells more effectively (American Cancer Society, 2019).

In a diagnostic, mAbs against specific cancer cell antigens are introduced into the patient's system to generate an immune response against the targeted cancer cell. This is a more specific method of cancer identification rather than tumour markers. MAbs that are able to recognise immune cell antigens have improved the diagnosis of certain types of leukaemia and lymphoma. MAbs are now diagnosing solid tumours such as carcinomas of the lung, breast, colon and rectum. In addition, special mAbs are used to diagnose colorectal cancer, breast cancer, lung cancer and ovarian cancer, such as the one that will be studied in this thesis.

To produce a mAb, the appropriate antigen to target must first be defined. Finding the right antigens for cancer cells is not always easy and, so far, mAbs have been shown to be more effective against some cancers than others. However, the cost of production remains high. This has made mAbs very expensive and unaffordable for many consumers.

The first mAbs in modern medicine were developed in the 1970s, when Georges Köler and César Milstein, working in a molecular biology laboratory in Cambridge, found a technique for making infinite quantities of antibodies with defined and predictable specificity, the hybridoma

technology shown in figure 1. This technique has revolutionised the study and practice of immunology and has also provided tools of immense value in many areas of biology and medicine (Sikora and Smedley, 1986).

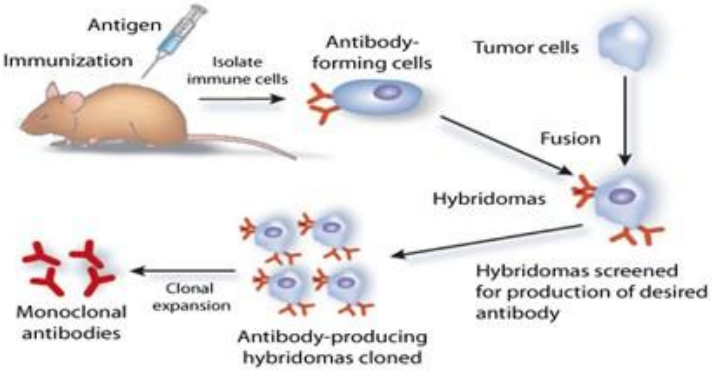


Figure 1: Schematic representation of the production of mAbs using hybridoma technology (Michnick and Sidhu, 2008).

Although their clinical potential was immediately clear, the development of mAbs into marketed products took some time (Kozlowski and Swann, 2006).

In 1986, following the market launch of the world's first antibody licensed for therapeutic use, muromonab-CD3 (Orthoclone® OKT3), a problem arose with these antibodies made exclusively with murine cells from mouse proteins, resulting in sensitisation of the recipients, which triggered an immune response when re-administered (PCSK9, 2015).

To prevent these potentially serious problems, and thanks to genetic engineering techniques, new molecular techniques were developed that resulted in second-generation mAbs, the recombinant mAbs depicted in Figure 2, in which parts of the murine antibody were replaced with human proteins (Castillo, 2017).

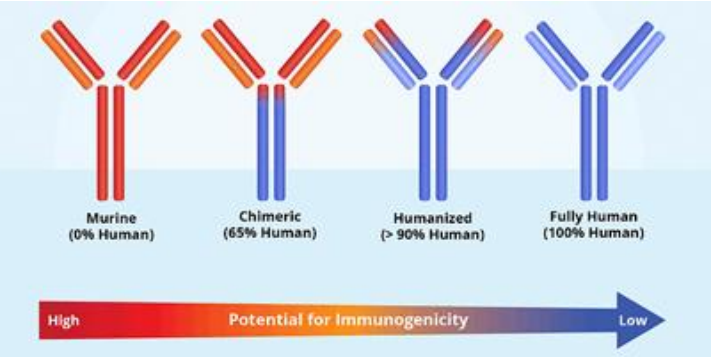


Figure 2: Types of mAbs according to their origin (Castillo, 2017).

In 1994, the Federal Food and Drug Administration (FDA) approved the second therapeutic antibody, Abciximab, which has a chimeric antibody fragment and constant regions with human sequences (Machado et al., 2006).

In 1997, the first humanised antibody, Daclizumab, was approved (Ruiz et al., 2007). Progress in the development of humanised mAbs containing only a small proportion of murine components led to a clear improvement in tolerability.

State-of-the-art biotechnological methods have led to the production of fully human antibodies. These antibodies have the lowest risk of triggering immune responses, which has made them a promising therapeutic alternative for a number of indications. In 2003, Humira® was the first fully human antibody to be approved in the United States for the treatment of rheumatoid arthritis (Kozlowski and Swann, 2006; Hwang and Foote, 2005; PCSK9, 2015).

The production of human mAbs against cancer cells has generated a great deal of interest given the difficulty of producing tumour-specific antibodies in animals.

MAbs are immunoglobulins of enormous value and interest since their production method makes it possible to manufacture unlimited quantities of a single antibody against a specially selected antigen. This not only makes possible many advances in our understanding of the basic principles of the proper functioning of the immune system, but also offers the possibility that such knowledge may be used to initiate the development of new diagnostic and therapeutic methods in the years to come.

MAbs represent a breakthrough for the treatment of many diseases, with particular interest for the cure of cancer, as their production method allows unlimited quantities of a single antibody against a specially selected antigen to be made.

MAb therapy is currently the fastest growing area in the pharmaceutical industry, constituting the largest group of biotech drugs. The ability to generate specific antibodies for a wide range of diseases is of great importance, yet their preparation is still too difficult. Therefore, the study of the preparation and design of a manufacturing plant can be of great interest.

2. OBJECTIVES

The generation of monoclonal antibodies was first described in 1975, but its development as a method of treating diseases has been slow, and the first treatments using monoclonal antibodies had to wait until the end of the last century. However, it is currently considered the most promising treatment for diseases such as cancer.

The preparation of monoclonal antibodies is carried out by means of cell culture and the application of numerous purification steps that avoid the presence of impurities that could compromise the efficacy of the treatment. The steps in the preparation must ensure the reproducibility of the product with respect to that used in clinical trials.

The objective of this work is to study the preparation of monoclonal antibodies, and to apply this study to the design of a monoclonal antibody manufacturing plant that ensures the reproducibility of the product. The tasks to be carried out are the following:

- Selection of a representative monoclonal antibodies for the study of their preparation.
- Compilation of applicable manufacturing regulations in the field.
- Synthesis of the manufacturing process for the selected product. Through a bibliographical study, the culture system will be stablished and the purification operations to be carried out, will be selected.
- Basic design of a multipurpose plant that allows the manufacture of the selected monoclonal antibody. The Aspen Batch Process Developer tool will be used for scaling the operations, selecting the equipment, and scheduling the production.

3. SELECTION OF A REPRESENTATIVE MONOCLONAL ANTIBODIES FOR THE STUDY OF THEIR PREPARATION

MAbs have become the current gold standard and one of the most promising treatments for cancer cure. That it is the current reference modality is no coincidence, but in part because mAbs fit easily into production and have demonstrated a lower safety risk in clinical trials compared to trials of other modalities, and they provide a fast track to new therapies or proof-of-concept studies (Ecker et al., 2015).

The competent health and drug organisations in the United States and Europe, the Federal Food and Drug Administration (FDA) and the European Medicines Agency (EMA) respectively, have already approved 97 therapeutic mAbs by August 2020, of which more than a third are for treating different types of cancer (Reichert, 2021).

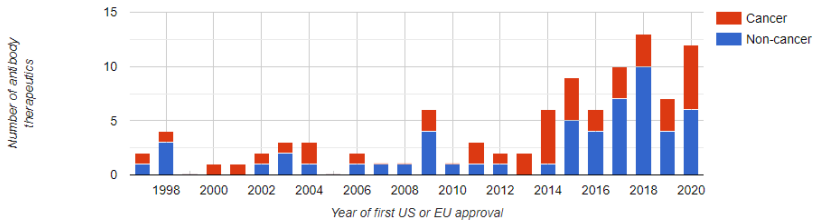


Figure 3: Number of antibody therapies receiving a first approval in the US or EU each year (Reichert, 2021).

As of 25 May 2021, 19 investigational antibody therapies are under regulatory review in the US or the EU.

Despite the pandemic and according to publicly available information, 44 mAb therapies are in late-stage clinical trials as of November 2020, although none of these are cancer-related, 6 of these therapies are for COVID-19 and 6 are planned for marketing applications in 2021 (leronlimab, tezepelumab, faricimab, ligelizumab, garetosmab and fasinumab) (Kaplon and Reichert, 2021).

We know that the mAbs sector has been booming for years and that is why the last 30 years have seen an impressive growth in sales of therapeutic mAbs products and an increase in demand for mAbs antibodies in the pharmaceutical industry market, such as Adalimumab (humira TM), bevacizumab (rituxan TM), rituximab (avastin TM) and trastuzumab (herceptin TM).

The organisations named above, FDA and EMA, are reviewing marketing applications for 16 investigational antibody therapies, which could make 2021 a record year for the number of approvals of mAbs. Of these 16 mAbs, five are potential treatments for cancer and eleven are potential treatments for non-cancer indications (Kaplon and Reichert, 2021).

The main motivation for further research in the field of therapeutic mAbs is the increasing number of cases of diseases such as cancer and autoimmune disorders. Cancer is caused by the abnormal proliferation of cells and one of the main problems in treating patients with cancer is inadequate assessment of the disease. There are two types of tumours: benign and malignant. The former, although they can grow at a considerable rate, remain localised at the site of origin and do not invade or affect adjacent organs, possibly leading to a full recovery in the patient's final diagnosis. Malignant tumours, on the other hand, are characterised by their ability to grow rapidly, invade adjacent tissues and organs, and spread through the blood or lymphatic system to establish what are known as metastases or new distant tumour colonies (Sikora and Smedley, 1986).

Indeed, what medicine needs to continue to grow and develop in the treatment of cancer are techniques that specifically detect tumours and that are safe, and that the clinician can use to discover whether his or her patient shows evidence of malignant disease. Techniques and substances to detect tumours already exist, these are often referred to as tumour markers and, in many cases, their place in clinical medicine is already well established. The targeting of these

circulating tumour markers has been greatly facilitated by the development of specific mAbs (Sikora and Smedley, 1986).

While it is relatively easy to construct a panel of antibodies that are active against a specific tumour, it is much more difficult to characterise the specificity and generate antibodies that react with a defined target antigen. The most common way to characterise mAbs is to study their binding to a set of different titre lines. Unfortunately, as the sensitivity of these assay methods increases, the apparent specificity often disappears.

Types of mAbs used to treat cancer (*American Cancer Society, 2019; Carvajal et al., 2019; Ruiz et al., 2007*):

- Pure mAbs are the most common type of mAbs used to treat cancer; they can work on their own without any drugs or radioactive substances attached to them. Most of them bind to antigens on cancer cells, but some work by binding to antigens on other non-cancer cells, or even free-floating proteins. They can work in different ways: some enhance a person's immune response against cancer cells by binding to them and acting as a marker for the human immune system to destroy them, some enhance the immune response by targeting immune checkpoints, and some bind to antigens on both cancer cells and other nearby cells that cause the cancer cells to spread or grow.
- Conjugated mAbs are either a chemotherapeutic drug (chemically labelled antibodies) or a radioactive particle (radioactively labelled antibodies) and work as a homing device to deliver the substance to the cancer cell. The mAbs expel the toxic substance once they encounter the target antigen on the cancer cell, thus reducing or avoiding any damage that may be caused to the rest of the cells.
- Bispecific mAbs are composed of two different mAbs, so they can bind to two different proteins at the same time. By binding to them, they bind to both cancer cells and immune cells, causing the immune cells to attack the cancer cells.

Some examples of different mAbs used for cancer treatment. Rituximab, Ibritumomab and Tositumomab are applicable in the treatment of lymphomas and B-cell neoplasms. Alemtuzumab treats chronic lymphocytic leukaemia and prevents kidney transplant rejection. Gemtuzumab and Alemtuzumab are therapeutic mAbs targeting leukaemia. Trastuzumab is

effective in breast cancer. Nimotuzumab and Cytuximab are used for the treatment of carcinomas (Garcia Calvo, 2016; American Cancer Society, 2019).

Another example of a mAb for cancer treatment is bevacizumab, a humanised mAb targeting human vascular endothelial growth factor (hVEGF). It is widely overexpressed in a variety of human solid tumours and plays a key role in tumour angiogenesis. (Chen et al., 2016).

In clinical practice it is used for the treatment of multiple cancers. It is effective in the treatment of metastatic colorectal cancer, breast cancer, lung cancer, renal cancer, fallopian tube cancer, primary peritoneal cancer and epithelial ovarian cancer (*European Medicines Agency, 2015).*

Bevacizumab is derived from murine vascular endothelial growth factor (VEGF). It contains 93% human and 7% murine sequence and has biochemical and pharmacological properties like the original murine mAb. It counteracts all hVEGF isoforms with high affinity and inhibits VEGF-induced endothelial cell proliferation and tumour angiogenesis, thus preventing tumour cell nutrition and debris exchange, as well as metastasis by preventing the spread of tumour cells in blood, but with reduced immunogenicity and a longer circular half-life compared to the murine antibody. Having the opportunity to inhibit VEGF, with drugs such as Bevacizumab, represents a breakthrough in cancer treatment (Gerber and Ferrara, 2005; García Calvo, 2016; Pedro Latorre, 2016).

Bevacizumab was developed by Roche/Genentech under the trade name Avastin. It was approved by the US FDA for clinical use for the treatment of various cancers in February 2004. It was approved for marketing by the EMEA (Europe, Middle East and Africa) in January 2005. It is currently used in various forms for the treatment of several tumours, including the treatment of an eye disease. A similar product from Pfizer has recently been approved (Cué Brugueras, 2004).

Although increasing evidence points to the high potency of bevacizumab for therapies of various diseases, today, there are still several barriers that restrict its widespread applications. One of these is the high cost of bevacizumab therapy, which is production-driven and relies exclusively on mammalian expression systems (Makino et al., 2011; Spadiut et al., 2013).

As can be seen it has breadth of applications for the treatment of different types of cancer and with many previous studies to be able to make a good study of its preparation process, with a personal interest in its application to ovarian cancer.

Next, we will study the possible manufacturing processes of the chosen antibody, Bevacizumab, and then make a basic design of a multipurpose plant that allows its manufacture.

4. COMPILATION OF APPLICABLE MANUFACTURING REGULATION IN THE FIELD

Over the past few years, the WHO (World Health Organisation) has approved a multitude of mAbs, including those used in cancer medicine. The agency remains focused on ensuring the quality of antibodies and facilitating the development of these products. Validations and controls on mAbs and their manufacturing processes have been and continue to be performed to update and comply with current regulations and for their proper development and performance as a product.

The validation of a process, procedure or method consists of verifying that it is suitable for an intended use, in addition to complying with all safety and environmental standards. Therefore, what is studied in validation is the ability of the process to do what it is supposed to do and to meet the established requirements.

Validation can be broken down into three main areas: medical usefulness and efficacy, product safety and proper operation of the facility (Ministry of the Environment and Rural and Marine Affairs, 2011).

In particular, our validation will be appropriate when it is demonstrated that: the mAbs manufactured meet medical expectations, consumer and environmental safety standards, and that the facility manufacturing these antibodies is safe and cost-effective (Ministry for the Environment and the Marine Environment, 2011).

In the pharmaceutical sector, the regulations to be followed for the manufacture of medicines are contained within good process practices (GxP), which include good manufacturing practices (GMP), good laboratory practices (GLP), good clinical practices (GCP) and finally good distribution practices (GDP) (Rodríguez, 2021).

We will focus on GMP, which are based on European regulations, royal decrees, regulations and the standards of the International Conference on Harmonisation (ICH), which set out the

guidelines to be followed for the correct manufacture of medical products, including mAbs (Rodríguez, 2021).

Table 1: Schematic of the validation of the mAb manufacturing process.

VALIDATION OF THE PROCESS			
REGULATORY COMPLIANCE (GMP, GLP, GCP, GDP)			
CONTROL OF THE MANUFACTURING PROCESS	Raw materials and their initial processing	Qualification and characterisation of the production process	Continuous process verification
CONTROL OF THE FINAL PRODUCT	Medical efficacy	Consumer safety	Economic efficiency
SAFETY CONTROL	Safety of the installation and storage	Staff security	Waste and environment

Table 1 shows schematically the parts and requirements of validation. The validation must take into account the regulations in force, as well as the aims and objectives of our project. Compliance with regulations is verified with the GxP and validation of compliance with our objectives is carried out through controls and tests.

VALIDATION OF THE MANUFACTURING PROCESS

Process controls include raw material acceptance criteria, in-process testing, defined set points and operating ranges for defined process and process parameters and lead times. Conditions for reprocessing or rework of intermediates must be evaluated and defined. The assays used for testing must be sensitive, reliable and take into account state and international regulations. Therefore, an important strategy used to control the process will be validation and compliance with current regulations (*Rezquellah, 2015; TCM, 2021; Tabuenca, 2014*).

Process controls include raw material acceptance criteria, in-process testing, defined set points and operating ranges for process parameters and defined holding times. Conditions for

reprocessing or reworking of intermediate products should be considered and defined. The tests used for testing must be sensitive, safe and take into consideration state and international regulations. Therefore, an important strategy to control the process will be validation and compliance with current regulations (Rezquellah, 2015; TCM, 2021; Tabuenca, 2014).

In general, validation involves repeating a step or test under a variety of defined conditions. Success in meeting specific criteria, elimination of impurities and compliance with standards are basic to process validation and demonstration that the process is under control (TCM, 2021).

For the correct validation of the process, the following control points must be taken into account (QBD, 2021):

- Raw materials and their initial processing: The drug raw material and its initial processing (harvesting for cell culture) can significantly influence the level and type of impurities that will later need to be purified, as well as significantly affect the quality of the final product.
- Qualification and characterisation of the manufacturing process: In this stage of process validation, the product design is analysed where it is to be identified from a collection of data whether it has a commercially reproducible behaviour.
- Continuous verification of the process: The validation of the process is not indefinite and must be constantly verified to be correct, during the production of the product a routine verification is maintained to corroborate that the state of the process is controlled, one of the ways of doing this is by means of the process control charts.

PRODUCT CONTROL

Product control or quality control is essential to know whether the specifications established by the product engineering are met and to assist the manufacturing department so that production meets these specifications. To control the quality of a product, inspections or sample tests are carried out to corroborate that the characteristics of the product are optimal, and a certificate of analysis is drawn up, which must be totally independent of production, and which contains the specifications and the results of the control.

Its function is to ensure that the products or services meet the minimum quality requirements in accordance with current regulations, the levels of impurities and the functionality that it must have. The certificate of analysis, which contains the specifications and results, is the first element to be examined to assess the quality of a batch. It exists mainly as a service organisation, for the knowledge of the specifications established by the product engineering and to offer assistance to the manufacturing department, so that the production complies with these specifications (Rezquellah, 2015; Tabuenca, 2014).

SAFETY OF THE INSTALLATION

The safety of the installation and its various parts is achieved through the application of GMP standards and their subsequent validation by means of continuous checks and controls. It is necessary to implement a system of self-inspections that allows companies to verify for themselves that the degree of application in compliance with the correct manufacturing standards is adequate and that also makes it possible to identify failures in these standards, facilitating the proposal of corrective measures (Farmaindustria, 2021).

The main standards necessary to comply with GMP are (*Rodríguez, 2021*):

- Staff: In order to carry out a correct elaboration, it is essential to have trained staff, with qualifications and experience, according to the position where they exercise their function, which allow them to carry out the process controls.
- Installations and equipment: The equipment used in the manufacturing or product elaboration process must be qualified and calibrated for its correct operation. In addition, all processes and procedures carried out within the manufacturing process (including reception of goods, storage and distribution) must be validated and correctly documented. The manufacturing or processing companies must have the necessary resources and infrastructures to be able to carry out the correct production of the products.
- Production, distribution and storage: Manufacturing processes must follow clearly defined procedures and conform to GMP in order to obtain high quality products. The storage of chemical products and their transport involve risks in themselves and in

contact with other materials. Incorrect or faulty storage can pose a danger to the managing company and its workers.

- Documentation: In order to maintain GMP, it is very important to keep records of all manufacturing processes. In addition, companies must have extensive documentation detailing each of the procedures carried out during the manufacturing process.
- Quality control: Quality control must be involved in all decisions during the manufacturing process that affect the quality of the product. One aspect, already mentioned above, which is fundamental to the success of quality control, is the independence of quality control from production.
- Outsourced activities and self-inspection: Companies that carry out manufacturing processes have a relationship with other companies, either through the purchase of material, the subcontracting of some process or service, etc. Therefore, in order to comply with GMP standards, it is important that these companies also follow the regulations that allow them to comply with these standards and thus do not negatively affect the quality and safety of the products.

STATE AND INTERNATIONAL ENVIRONMENTAL REGULATIONS

In order to verify the correct functioning of the systems for the prevention, correction and monitoring of air pollution, emission limit values, and the conditions established in the permit and in the applicable regulations on air pollution, it is necessary to carry out an adequate inspection and control.

These measures can be carried out at three levels (*AEMPS, 2020; Sede Electrónica de la Agencia Española de Medicamentos y Productos Sanitarios, 2021; European Medicines Agency, 2021*; *Farmaindustria, 2021; Diario oficial de la UE, 2015; Drager web, 2021*):

- External control of emissions, carried out by the control bodies established by the autonomous community, where it includes the verification and checking of:
  - The conditions established in the authorisation and in the applicable regulations on atmospheric pollution.

- Operation of the systems for the prevention, correction and monitoring of atmospheric pollution.
- Emission limit values.

- The internal control of emissions, carried out by the person responsible for the installation in accordance with the criteria and by the means determined by the competent administration. It also includes the checking and verification of the same elements as in the previous case.
- Inspection by the services of the Autonomous Communities, which once again includes the elements dealt with in the first point.

REGULATIONS TO BE TAKEN INTO ACCOUNT IN OUR INSTALLATION

There are a number of regulations that must be taken into account in our installation (*AEMPS, 2020; Diario oficial de la UE, 2015; Ministerio de medio ambiente y medio rural marino, 2011*):

- Principals
  - Directive 2003/94/EC on medicinal products and investigational medicinal products for human use.
  - Article 26 of Law 34/2007 - Regulation of inspections on atmospheric emissions.
  - Royal Decree 258/2019, and Royal Decree 824/2010 - Regulation of pharmaceutical laboratories. This Royal Decree has been totally or partially repealed by Royal Decree 824/2010 of 25 June 2010, which regulates pharmaceutical laboratories, manufacturers of active pharmaceutical ingredients and foreign trade in medicines and investigational medicinal products, which, in its Chapter IV, includes the principles and guidelines of GMP.
  - The EU GMPs for medicinal products and active substances are published as volume 4 of the EU-Eudralex legislation.

- Periodically, the Spanish Agency for Medicines and Health Products (AEMPS) of the Ministry of Health, Social Services and Equality translates and publishes any updates to Volume 4 of the GMP.
- Compliance with GMP.
- Catalonia - Law 22/1983, of 21 November 1983, on the Protection of the Atmospheric Environment.
- Decree 203/2009 of 22 December 2009 and Decree 152/2007 of 10 July 2007 extending the Action Plan for the improvement of air quality in municipalities declared special protection areas for the atmospheric environment.
- Decree 80/2002 of 19 February 2002 regulating the conditions for waste incineration.
- Decree 199/1995, of 16 May 1995, approving the vulnerability and capacity maps of the territory with regard to atmospheric pollution.
- Decree 322/1987, of 23 September, on the development of Law 22/1983, of 21 November, on the Protection of the Atmospheric Environment.
- Royal Decree 117/2003. Calculation of fugitive emissions of particles in extractive activities.

- <u>Other regulations to be taken into account</u>
  - Obligations of pharmaceutical laboratories in relation to the formal risk assessment of active ingredients for the manufacture of medicinal products for human use.
  - Registration, annual declaration of activities, inspection and certification of manufacturers, importers and distributors of active ingredients.
  - Guidance on the content of the technical memory for the pharmaceutical industry.

There are also GMP standards for pharmaceuticals and additives proposed by the ICH, and their scope covers the countries that make up the ICH (the European Union, Japan and the United States), although other countries, such as Australia and Canada, also adopt them.

REGULATORY DISCUSSIONS

The time from investigational new drug (IND) submission to licensed product has ranged from more than 13 years to less than 5 years. Most licensed antibodies have undergone single cycle reviews.

Areas of product failure align with the three dimensions of the critical path: medical utility (efficacy), safety, and industrialisation (manufacturing and quality). A frequent reason for product failure is that the product does not show efficacy in clinical trials. While ideally this failure would be detected early in development, in many cases, it has taken a pivotal study to reveal the lack of efficacy. Choosing the right indication is critical and successful products have failed in their initial indication.

Even if the product works in the chosen indication, poor study design may not reveal or support real benefits. Safety issues arising from clinical trials can stall or delay product approval. Although manufacturing problems are not usually the cause of product failure, manufacturing problems can significantly delay product approval for complex biotech medicines. Lack of manufacturing consistency or the initiation of manufacturing changes late in product development has delayed approval of mAbs products.

Many mAbs products have been successfully manufactured and marketed using a combination of process validation, process control and product testing. There are a number of common pitfalls in early antibody development that can be avoided with proper planning and guidance. Advances in specification and manufacturing approaches can accelerate the industrialisation of mAbs and other biotechnology products. The key to using these approaches is to define critical product attributes and link them to critical process parameters. Product complexity and heterogeneity suggest that critical product attributes can only be defined with a better understanding of product biology and structure-function relationships.

5. SYNTHESIS OF THE MANUFACTURING PROCESS FOR THE SELECTED PRODUCT

Due to a growth in demand for mAbs products, production capacity had to be increased through the construction of large bulk manufacturing plants as well as improvements in cell culture processes (*Brian Kelley, 2009*).

Antibodies are soluble proteins, which are part of specific immunity and are made up of combinations of two light chains and two heavy chains linked by disulphide bridges. The area of the antibody responsible for specific binding to the antigen is called Fab (Fixation antigen binding) and the remaining fragment is called Fc (crystallisable fraction); this nomenclature will be used later to explain the production and synthesis process (*Calvo, 2016*).

The production of mAbs is being improved by protein engineering and at the same time, their therapeutic profile is being improved. The processes for manufacturing recombinant therapeutic mAbs have several common characteristics and consensus has emerged among the major biopharmaceutical process development groups to standardise their manufacturing process and especially the production of purified bulk drug substance, i.e., the intermediate used to produce the final drug product (*Kelley, 2009; Jefferis, 2009*).

It stands to reason that, if the manufacturing processes for different mAbs share many of the same production characteristics, the similarities will outweigh the differences when comparing different process flowsheets and typical manufacturing plant layouts. Although each company uses a slightly different platform process, it has been reported that they have very similar development timelines from cell line inception to early clinical trials (*Kelley, 2009*).

For common benefit and advancement, many companies have standardised and matured their mAbs process technology, and not only that, but joint tools such as high-throughput systems are being used for cell line development and purification processes. This common

production technology provides a high degree of robustness and also makes the results of product quality, production capacity and costs predictable (*Kelley, 2009*).

In recent years, the productivity of mammalian cell culture processes fed by mAb batches has increased significantly due to two key factors. The first is that there is now a better understanding of chemically defined media and also of feeding strategies to achieve sustained viability while the bioreactor is running. The second key factor is that the cell lines have adequate growth and high specific productivity (Qp) (*Birch and Racher, 2006*).

Mammalian cells are used for the expression of all commercial mAb therapeutics and are grown in suspension culture in large bioreactors. The biological systems that are used industrially (biofactories) are NSO cells (murine myeloma cells) and CHO cells (Chinese hamster ovary cells). CHO cells are the ones used in the biotech industry because of their versatility and high yield, but they cause tolerance problems in patients. They have attractive process performance attributes, such as rapid growth, high expression and the ability to adapt for growth in chemically defined media (*Brian Kelley, 2009*).

Initially recombinant cell culture processes for antibody production had low expression levels. Advances in recombinant technology based on cloning and expression of heavy and light chain antibody genes in CHO cells led mAb production groups to take advantage of common technologies already in use for more robust and mature recombinant products (*Birch and Onakunle, 2005*).

The first recombinant mAbs were in high demand in the market and had a combination of low titres, which led companies and organisations to build large production plants with several bioreactors. This in turn, together with improvements in production processes, led to higher expression results and cell densities, ergo, they could be combined to provide higher product titres (*Kelley, 2009*).

The synthesis and production process explained below consists of two main phases. On the one hand, there is the upstream phase, which is based on small-scale production and is where the culture is prepared for large-scale processing; this phase can also be defined as a series of stages for cell expansion. On the other hand, there is the downstream or transformation phase,

which is based on large-scale production with very large volumes, in this phase the stages of purification and recovery of raw material stand out (*Castillo, 2017*).

Figure 4: Key steps in the production process of a mAbs (Castillo, 2017).

Both upstream (process steps associated with the production of a recombinant protein by culture and propagation of host cells) and downstream (process steps associated with the purification of a recombinant protein and removal of impurities) process platforms have become widely established in industry to meet the need for production processes that can be rapidly developed to produce large quantities of pharmaceutical-grade mAbs in a consistent and reproducible manner at moderate costs (*Jefferis, 2009*).

Large-scale production of mAbs uses mammalian production systems followed by cell removal and purification through sequential chromatographic and membrane filtration steps to consistently reduce product and non-product impurities to acceptable levels (*Shukla and Thömmes, 2010*).

UPSTREAM

The upstream phase begins with the cultivation of the cells that will produce the therapeutic protein. These cells are cultured on a small scale, in flasks or Petri dishes, which contain the

culture medium necessary for the cells to grow. Once the cell line is obtained, it is cryopreserved in numerous vials to create a cell bank. When the batch manufacturing process is to be carried out, a vial from the cell bank is thawed and cell culture is started in a flask containing a small volume of culture medium (*Castillo, 2017*).

During the upstream process, the cells are sequentially transferred to larger and larger vessels containing larger volumes of culture medium. This culture development process contains four reactors from smallest to largest volume. The smallest changes can affect the cells and alter the proteins they produce. Therefore, strict controls are required to ensure the quality and reproducibility of the final product. Variables such as: temperature, pH, oxygen level, nutrient concentration, etc. must be controlled. In addition, tests must be carried out to check the absence of contamination with bacteria, yeasts or other micro-organisms, as any contamination of a culture will spoil the whole batch. These controls are carried out manually (*Castillo, 2017*).

Next, the cells that have shown the greatest capacity for stable growth over several cultures are cultured in higher-volume equipment and their capacity to produce the protein of interest and the quality of this protein is assessed, based on its correct structure, glycosylation, functionality, etc. This stage is very critical, as it must end with the selection of the master cell line, which will be the one to be definitively transferred to the last stage of the process (*Castillo, 2017*).

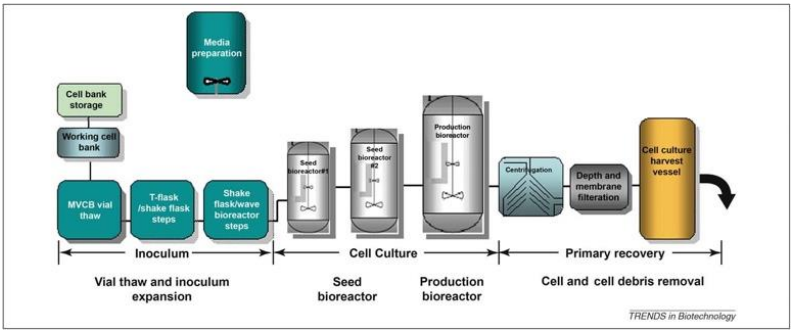


Figure 5: Schematic diagram of an upstream production process often used for the production of mAbs and other glycoproteins (Shukla and Thömmes, 2010).

To lay the foundation for the development of the cell culture production process, a fast-growing and highly productive specific production cell line must be created. Furthermore, additional requirements such as the ability to grow in chemically defined media, the stability of the cell line and the use of efficient expression systems must be taken into account (*Birch and Racher, 2006; Chusainow et al., 2009*).

The first stages of inoculum growth after vial thawing in large-scale culture processes occur in shaker flasks or spinner flasks, which progressively increase in size and thus volume. The cell mass is then scaled up through several stages in the bioreactor and transferred to the production bioreactor. The most common production is fed-batch production and involves the addition of small volumes of feed to supplement and complement the nutrients present in the bioreactor as product production and cell growth progresses (*Crowell et al., 2007; Chee et al., 2005*).

There are several factors that must be controlled during cell growth in the production bioreactor for proper cell growth, including dissolved oxygen, temperature, pH, O_2 transfer and CO_2 transfer (*Crowell et al., 2007; Chee et al., 2005*).

A high level of product synthesis in CHO cells has been linked to increased gene copy number and efficient transcription and thus stability (*Chusainow et al., 2009*). Cell lines used for antibody expression include a multitude of CHO cell lines, the most common being murine hybridoma, murine lymphoid (e.g., NS0 and SP2/0) and human PER.C6.

To arrive at the best combination of properties, a large number of clones need to be generated and analysed. Cell lines belonging to the production are screened during manufacturing to select those with high specific productivity (Qp), although not all cell lines with high productivity will perform well in our production process. Stability profiles and desired product quality metrics must also be taken into account. Several automated systems are currently used to facilitate the identification of the most suitable production line through screening (*Jain and Kumar, 2008*).

One of the techniques used to select cell lines with high levels of antibody production is fluorescence-activated cell sorting. At the same time, for the identification of the most suitable clones for antibody production, there is a technique that uses relative mRNA transcript levels to

predictively detect high expressors, using the ratio of light to heavy chain mRNA levels as a predictor of the levels of aggregated products. Another characteristic that we monitor is the growth of clones, which is usually controlled by early high-throughput screening in 96-well plates in addition to some level of screening in shake flasks or even small bioreactors (*Lee et al., 2009*).

During pre-clinical development stages, transfectant pools are frequently used to produce significant amounts of product quickly, in addition to the generation of material through cell line development. Their use at scales larger than a few hundred litres has now been discarded in favour of isolating the most desirable subset of clones for biopharmaceutical production early in the clinical development process (*Haldankar et al., 2006; Ye et al., 2009*).

Perfusion cell culture, in which cells are retained in the bioreactor and new media are continuously fed, is used in some cases only because of its great challenge to maintain the sterility of the bioreactor for long periods of time. One of the most important advances in achieving higher titre cell cultures has been the design of serum-free media with strict and defined chemistry, which can support both product secretion and cell growth. Another key advance has been the improved understanding of nutrient limitations during cell culture and being able to provide a response to this (*Crowell et al., 2007; Chee et al., 2005*).

In large-scale processes, the use of whey has been effectively replaced with hydrolysates from plant sources or yeast, which are often key components of cell culture media. Amino acids and trace media constituents such as metals have been shown through development studies to significantly influence product yield in production bioreactors (*Crowell et al., 2007; Chee et al., 2005*).

The ability to maintain gas exchange efficiency is a key criterion to ensure the success of cell culture platforms. For this reason, bioreactors have also been developed for the optimisation of gas exchange, oxygen under-supply to maintain growth, cell productivity and CO_2 removal (*Matsunaga et al., 2009*).

For the collection of the mAb cell culture, one of the typical procedures is centrifugation followed by depth and membrane filters to remove cells and cell debris prior to subsequent purification. These depth filters consist of a fibrous bed that can trap particles within its volume,

unlike membrane filters that do not trap but reject them. In addition, depth filters have recently been shown to have different adsorption properties for soluble impurities, such as host cell proteins (HCP) or DNA, in addition to their primary function of particle removal filtration. Therefore, the choice of depth filter type and operating conditions must take into account the subsequent purification process in an integrated manner (*Yigzaw et al., 2006*).

DOWNSTREAM

As mentioned above, the post-processing phase is the downstream or large-scale production phase, i.e., at the scale of production required, which will be determined by production needs (based on the volume of the market for which the drug is intended) and the productivity of the cell line (*Castillo, 2017*).

Once the culture and cell line have been created, the mAb is isolated from the cells and then subjected to successive filtration and purification processes (chromatography, ultrafiltration, conditioning, etc.). These processes are also a very important part of the manufacturing process, as they can have a critical influence on the overall economy of the process depending on the yield obtained. Finally, the product is formulated according to current specifications and conditioned for clinical use (*Castillo, 2017*).

In documents, studies and projects consulted to understand the downstream process and to carry out this work, differences have been observed when it comes to defining the end of the downstream process. The different experts in the field differ, as some consider the end of the process to be the concentration of the product once ultrafiltration has been completed, while others establish the end of this process one step further, in the sterilisation of the mAb once concentration has been completed.

All the steps that have been defined for the production of a mAb are drug specific. Any minor alteration can lead to changes in the structure of the active substance, compromising its stability or therapeutic behaviour, or increasing the risk of adverse effects. The parameters defining the manufacturing processes must be well specified for a correct assessment of product quality (*Castillo, 2017*).

It is very difficult to realise the platform approach using a templated process, even within the same company, due to the variability in behaviour and purification properties between different mAbs. Still, the platform helps us to achieve alignment and a common philosophy on the type of unit operations to include in contiguous processes (*Shukla et al., 2007*).

In large manufacturing plants it is common to find multiple bioreactors supplying one or sometimes two purification trains. Purification-related operations can often be completed in a single day, and therefore several bioreactors can be combined with the output of a single purification train (*Kelley, 2009*).

Critical points of purification are identified by assessing the process that suits a production facility. In addition, process designs that can be adapted to enable purification of larger batches can also be identified. Often, to improve on the aspect of the ability to purify larger batches, no new technology is required, simply changes and adjustments to the process that will avoid typical plant limits such as product group tank volume, process solution supply or unit operating cycle time (*Kelley, 2009*).

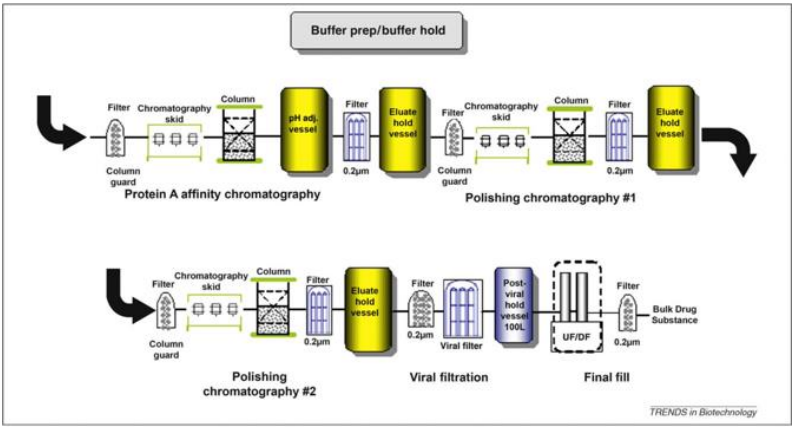


Figure 6: Schematic diagram of a typical downstream process, including other steps in the filtration process and aimed at viral clearance (Shukla and Thömmes, 2010).

The antibody purification process is initiated by collecting the contents of the bioreactor using industrial continuous disc stacking centrifuges followed by clarification using membrane and depth filters. Using protein A chromatography, the mAb is captured and purified, this

process includes a low pH elution step that also serves as a viral inactivation step. To meet purity specifications, two additional chromatographic polishing steps, anion exchange chromatography (AEX) and cation exchange chromatography (CEX), are usually required. To acquire additional assurance of viral safety, we perform a virus retentive filtration step, and a final ultrafiltration step that formulates and concentrates the product (*Kelley, 2009; Shukla et al., 2007*).

As a capture step in the process, all schemes rely on the use of protein A affinity chromatography. This affinity chromatography method is based on the specific binding affinity between the Fc region of the mAb and the protein A ligand. This specificity allows HCP, DNA and other impurities from the cell culture process to flow away while the product binds to the stationary phase, providing high purity in just one step. For the elution of protein A affinity sorbents to be effective, we must have a relatively low pH. The high specificity of the protein A step has facilitated the adoption of a platform approach for downstream purification of mAbs (*Shukla and Thömmes, 2010*).

The purification of nuclei in industrial bioprocesses, traditionally performed by batch chromatography, has long been a laborious and time-consuming process requiring large volumes of mobile phase and resulting in very large pool volumes. In recent years, advances have been made in the design of chromatographic stationary phases for the purification of biomolecules, which have provided some solutions to the problems encountered with the throughput of the facilities and have facilitated the development of more productive platforms for the industrial purification of proteins and in particular, mAbs (*Ghose et al., 2004*).

To ensure product safety, host cell proteins must be reduced to low levels as they constitute an important class of process-related contaminants. On the one hand, the protein A capture step is known to be highly selective for mAb over soluble host cell protein impurities, although the levels of host cell protein that often persist after purification can still pose a clean-up challenge for the affinity-free polishing steps (*Shukla and Thömmes, 2010*).

One of the problems in this part of the process is that HCP species that persist during the protein A step often associate with the product species. It has been shown that these protein-protein interactions are disrupted by chaotropic combinations at high pH where mAb-Protein A

interactions remain strong. This solves the problem as it has allowed the development of selective washing conditions that can reduce host cell protein levels after protein A chromatography (*Sukla and Hinckley, 2008*).

To validate this part of the process, multiple strategies, including worst-case, bypass and spiking studies, have been employed to demonstrate robust removal of HCP by a downstream mAb process. The low pH conditions used for protein A elution often result in precipitation problems for the product species. These have been shown to be mitigated by judicious buffer selection or by selection of stabilising additives for the elution buffer (*Shukla et al., 2007*).

It has also been shown that turbidity is caused, in some cases, by precipitation of contaminants from the HCP and not from the product, which is indispensable from a filtration and process reproducibility perspective. A solution to this problem has recently been found and is the proper sizing and design of the collection depth filtration stage. Some platforms adopt a completely template-based approach to the polishing steps AEX and CEX, while others have a molecule-specific approach based on the particular impurity clearance challenge (*Yigzaw et al., 2006; Kandula et al., 2009*).

The anion exchange column is generally operated under continuous flow conditions with fairly high loading capacities. CEX column loading is often performed at low salt concentrations. mAb have a basic pH and the initial molecules adsorbing on the resin are usually at the entrance of the pores of the resin structure, which repels more protein molecules and restricts the loading capacity of the column. If resolution of impurities, particularly HCP and aggregates, is needed, the anion exchange chromatographic stage can be operated in a weak partition mode as opposed to actual flow conditions, resulting in retention of impurities (*Zydney et al., 2009*).

The use of smaller pores provides additional viral retention to what was already in place, although this has the risk that filters become clogged by small levels of particles that are not detected by analytical size exclusion chromatography. Viral filters are typically single-use filters and can be among the most expensive consumables in the process based on their cost per gram of product. In addition to proper placement of these filters in the downstream process, pre-

filters can be used to protect the viral filters from flow decomposition arising from high particle loading (Ireland et al., 2005; Bohonak and Zydney, 2005).

To arrive at the correct selection for a process, viral filters from various suppliers are typically examined, and in recent years there has been significant innovation in the development of low contamination viral filters that can still provide excellent viral removal. These filters are made of hydrophilic materials that reduce the tendency of proteins to unfold on the surface, thus minimising pore contamination. At the same time, further improvements have been made in pore morphology, with a reduction in the polydispersity of pore sizes. These have led to a progressive improvement in maintaining high fluxes with mAb process flows (*Marques et al., 2009*).

Another engineering challenge is the large production quantities of mAb and the handling of the large volumes of drug substance produced. The generation of such high protein concentrations requires special attention to the design of the UF (ultrafilter) / DF (diafiltration) step that is normally used for buffer exchange in the formulation buffer. The air-water interfaces created during pumping of such concentrated protein solutions can lead to product aggregation during large-scale production (*Bee et al., 2009*).

The flow path in the UF system is designed to minimise system hold-up volumes and decrease the incidence of areas where air entrainment could occur. For high concentration formulations, the concentration at which DF is performed is selected to minimise the number of pumping passes to which an average protein molecule could be exposed during the passage. Even exposure to stainless steel surfaces for significant periods of time can cause aggregations (*Bee, et al., 2010*).

If the drug substance requires freezing conditions during long-term storage, the design of freezing and thawing steps must be approached carefully to avoid changes in product quality caused by cryoconcentration and exclusion effects caused by slow freezing rates in large volumes, and denaturation of the product into large surface area ice crystals caused by fast freezing rates (*Bee et al., 2010*).

One of the factors that has created greater efficiency in the process development and manufacture of mAbs has been the adoption of a platform approach. Evolving the platform

through accumulated experiences while addressing the challenges faced by atypical antibody processes is an equally essential part of creating a robust and broadly applicable platform (*Shukla and Thömmes, 2010*).

For modern biopharmaceutical purification processes, chromatography has emerged as an essential pillar due to its high resolution and selectivity. On the other hand, however, the performance of conventional preparative chromatography has limitations due to the discontinuous nature of the process and is also limited in speed for medium-sized particle beads (*Shukla and Thömmes, 2010*).

Because of the high titres that can now be achieved in cell culture, it is believed that, in the future, the need to build new facilities with very large bioreactors will decrease, although current facilities will remain in use. Lower production volumes will increase the need for flexibility in facilities and faster turnaround leading to a growth in the use of disposables in manufacturing. This is why, although the current process platform is safe in practice, we believe that several drivers will lead to significant evolution over the next decade (*Shukla and Thömmes, 2010*).

Biopharmaceutical companies are becoming increasingly involved in the development of mAb products, and platform approaches are becoming more established. It is believed that the alternative technologies that will be used and applied first will be those that fit into existing bio-manufacturing facility designs, rather than requiring significant investment in the front end of the facility. Platforms have enabled significant efficiencies in process development from a time and resource perspective. Allied areas of biopharmaceutical companies, such as quality and manufacturing, have also benefited through the creation of templates for their documents and procedures (*Shukla and Thömmes, 2010*).

To conclude the novel advances cited in the preceding paragraphs and the various explanations of purification and residue treatment, it can be said that Protein A-based sorbents have been shown to be remarkably stable over many hundreds of cycles of use, providing high reliability for the Protein A chromatography tool in the antibody purification platform. In some process development groups, continued advances in cell culture technology have raised mAb titres steadily, putting pressure on purification technology that would normally limit or block plant production capacity. Concerns have also been expressed about the need to increase production

capacity and pressures to further reduce cost of goods sold (COGs). These factors could drive the development and implementation of new bioprocess technologies, such as perfusion technology for cell culture, or unconventional purification methods such as precipitation, crystallisation, continuous processing or the use of membrane adsorbers (*Low, 2007; Brorson, 2003*).

These factors and advances have resulted in excess manufacturing capacity and, together with improvements in conventional purification technologies, promise almost unlimited production capacity for the foreseeable future. The increase in titres has also led to a marked reduction in production costs, which could become a relatively small fraction of the selling price of future products that are sold at prices similar or close to current levels. Capacity reduction and cost pressures for next-generation bulk production processes may shift the focus of process development efforts and have important implications for both plant design and product development strategies for biopharmaceutical and contract manufacturing companies (*Kelley, 2009*).

ENFORCEMENT AND SAFETY IN THIS PROCESS

In this section, we will relate the GMPs described in the section on Regulations and validation of the mAb manufacturing process, with the manufacturing process that includes the synthesis of the preparation process of the selected products, the culture system and the purification operations. This relationship between the regulations and the manufacturing process only includes the most basic and important points to take into account in our process, as there are a wide variety of restrictions and indications that will have to be interpreted more thoroughly when constructing and designing our process.

- Preparation and selection of raw materials
  - Each batch of raw material, biological product, shall be tested against the specifications for that raw material prior to its use in manufacturing.
  - No batch of raw material shall be used in the manufacture of mAbs unless that batch of material complies with the specifications for that raw material.

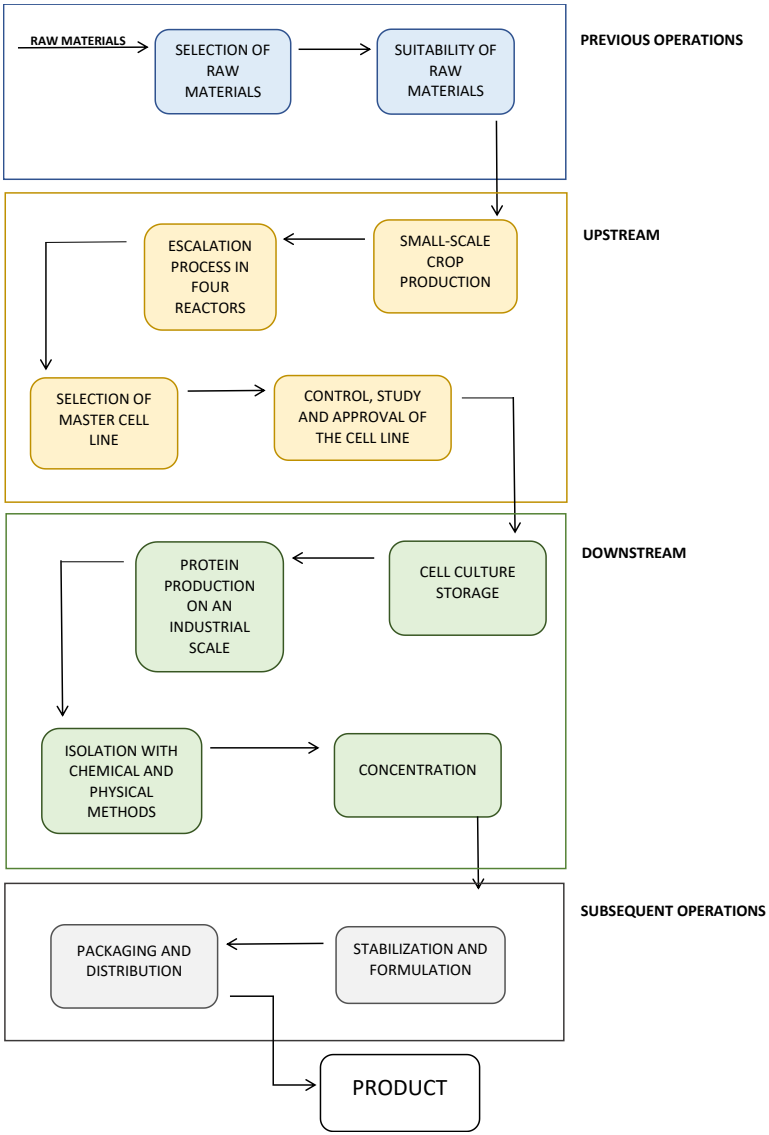
- It will be strictly necessary to ensure the correct storage of raw materials in tanks of appropriate size and shape, and their correct preservation so that no unexpected changes in the raw material occur during storage. In our case we must be strict with this point as our raw material will be very sensitive to small changes in pH, temperature and/or concentration.
- Before its use in the manufacturing process, it will have to be verified that its state is correct, especially the concentration of cultures, but also the pH and temperature, and that its specifications have not changed during storage.
- When the above specifications are not prescribed, they must: be in writing, be approved by those responsible for the process and the product quality area and be approved by the manager.

- Upstream and downstream process
  - Facilities must allow operations to be carried out in clean, sanitary and orderly conditions. We must be strict with the cleanliness of the reactors where the crop is produced (upstream), as poor sterilisation of these could negatively affect our next crops. We must also take into account the hygiene and cleanliness of the piping that links the unit operations together and ensure that no corrosion or blockage of the piping occurs.
  - Equipment must allow effective cleaning of all surfaces and also avoid contamination of the drug and the addition of foreign material to the product. Downstream tanks, reactors, catalysts, centrifuges and filters must also meet hygiene and safety standards.
  - The unit operations of the downstream process (purification, ultrafiltration, chromatography) must be properly validated, described and approved to comply with the constraints and meet our objectives.
  - The different steps, including the two process steps (upstream and downstream) must meet their functionality and performance strictly, otherwise solutions will

have to be found to make our process as cost-effective as possible in addition to meeting its functionality.

- The flow of materials through the installation must be designed to avoid mixing or contamination.

The steps to follow for the production of the different mAbs are shown schematically below.



6. BASIC DESIGN OF A MULTIPURPOSE PLANT THAT ALLOWS THE MANUFACTURE OF THE SELECTED MONOCLONAL ANTIBODY

In the previous chapter, a process for the preparation of mAbs was synthesised, focusing on Bevacizumab, a humanised mAbs targeting hVEGF.

This chapter, dedicated to the basic design of the production plant, will use the Aspen Batch Process Developer tool that facilitates equipment selection, batch time and cycle time calculation, and the scheduling of production campaigns.

The factors that will affect the operation of the processes are the processing time required to obtain a batch; the size of the batch; the possible overlapping of batches, which will condition the time required between batches; the storage policy, which will condition the starting times of the operations; and, finally, the organisation of production (*Gutiérrez, 2021*).

The process consists of two fundamental phases, upstream and downstream. In the previous section, we explained what this process consisted of by explaining the respective phases until the desired mAb was obtained. Now we will explain in more detail each of the steps, the volumes of the equipment, the operation times, the exact amount of material that enters and leaves in each of the phases...

UPSTREAM

This phase consists of the production of a large-scale culture from a small-scale production using four reactors in series and subsequent centrifugation.

Four reactors are used in series for future large-scale purification. The first three reactors (R-100, R-400, R-2500) are loaded and fermented twice and have capacities of 100L, 400L and

2500L respectively. The fourth reactor (R-12500) is the production reactor, has a capacity of 12500L and is loaded and fermented 3 times.

From R-12500 it is transferred to the centrifuge (C-1500) and then the mother liquor, called mAb solution, is sent to the final 12500L tank (T-12500).

INOCULUM 1 (R-100 → R-400)

CHARGE	FERMENT (2 DAYS)	CHARGE	FERMENT (2 DAYS)
30 kg Water 10 kg Feed 10 g CHO	$CHO_{(S)}$ = 3 kg $MAb_{(l)}$ = 0,2 kg Total amount: 40,0805 kg	30 kg Water 10 kg Feed	$CHO_{(S)}$ = 6 kg $MAb_{(l)}$ = 0,4 kg Total amount: 80, 033 kg

INOCULUM 2 (R-400 → R-2500)

CHARGE	FERMENT (2 DAYS)	CHARGE	FERMENT (2 DAYS)
60 kg Water 20 kg Feed	$CHO_{(S)}$ = 12 kg $MAb_{(l)}$ = 0,8 kg Total amount: 160,292 kg	120 kg Water 40 kg Feed	$CHO_{(S)}$ = 24 kg $MAb_{(l)}$ = 1,6 kg Total amount: 320,102 kg

INOCULUM 3 (R-2500 → R-12500)

CHARGE	FERMENT (3 DAYS)	CHARGE	FERMENT (4 DAYS)
480 kg Water 120 kg Feed	$CHO_{(S)}$ = 69 kg $MAb_{(l)}$ = 4,6 kg Total amount: 921,867 kg	800 kg Water 200 kg Feed	$CHO_{(S)}$ = 144 kg $MAb_{(l)}$ = 9,6 kg Total amount: 1920,68 kg

PRODUCTION (R-12500 → C-1500)

CHARGE	FERMENT (6 DAYS)	CHARGE	FERMENT (5 DAYS)
1600 kg Water 400 kg Feed	$CHO_{(S)}$ = 294 kg $MAb_{(I)}$ = 19,6 kg Total amount: 3930,1 kg	3200kg Water 800 kg Feed	$CHO_{(S)}$ = 594 kg $MAb_{(I)}$ = 39,6 kg Total amount: 7925,36 kg
CHARGE	**FERMENT (5 DAYS)**		
3200 kg Water 800 kg Feed	$CHO_{(S)}$ = 894 kg $MAb_{(I)}$ = 59,6 kg Total amount: 11920,6 kg		

HARVESTING (C-1500 → T-12500)

CENTRIFUGE
The centrifuge speed: 1000rev/min Transfer rate: 3 cubic m/h Deliquoring: 3h Speed of rotation: 100 rev/min Moisture content: 30%

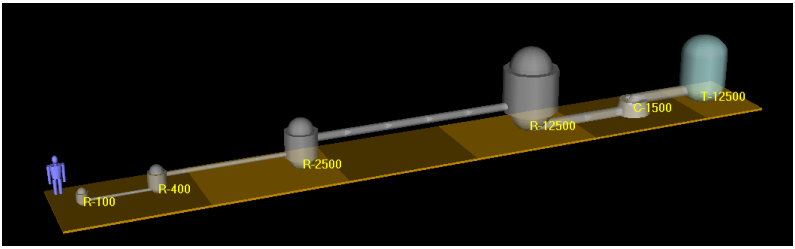


Figure 7: Simulation in Aspen Batch Process Developer of the upstream process.

All suppliers work on a customised design and construction of each individual piece of equipment, which means that the same supplier can offer a variety of different dimensions and conditions for the same piece of equipment.

All the equipment has been selected from local suppliers. As mentioned above, the upstream process consists of six different pieces of equipment, four reactors, one centrifuge and one tank.

A list of the equipment used in the upstream process with their respective local suppliers can be seen in table 2 below (Aguilar y Salas, 2021; De Dietrich, 2021; Comquina Europe, 2021).

Table 2: Upstream equipment suppliers

EQUIPMENTS	SUPPLIERS
Reactors	Aguilar y Salas
Disc centrifuge	Comquima
Tanks	De Dietrich

The complete recipe, for the simulation of the upstream process, as introduced in Aspen, can be found in the annexes.

Gantt diagrams are used to represent the production schedule, indicating the occupation of the equipment at any given moment.

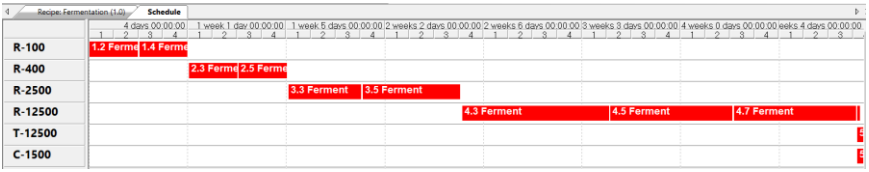


Figure 8: Estimated time of 1 batch expressed in a Gantt chart.

Figure 8 shows that the estimated production time for a batch is 31 days, speaking only of the fermentation process.

The time of each operation is a factor to be considered when assigning operations to equipment, as it will determine the equipment occupation times. The batch time or equipment occupancy time conditions the manufacturing time of a batch, thus affecting the number of batches that can be manufactured in each period of time (*Gutiérrez, 2021*).

If the batches are processed consecutively, starting the processing of a batch when the processing of the previous batch is finished, by multiplying the number of batches by the time of a batch, the time to manufacture a given number of batches can be calculated. In the case of Bevacizumab, approximately 10 batches could be manufactured in a year, given that a year has 365 days and there is a time margin.

Figure 8 shows that the equipment is idle most of the time. Of the 31 days it takes to manufacture a batch, the R-100 and R-400 units are idle for 27 days; the R-2500 unit is idle for 24 days, and the R-12500 unit for 15 days.

It is possible to think of schedules with higher equipment occupancy and, therefore, requiring less time to manufacture a given number of batches.

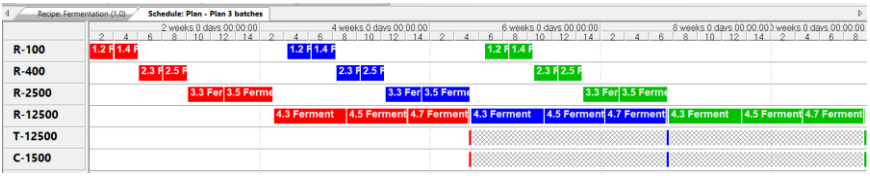


Figure 9: Estimated time of 3 batches with overlaps expressed in a Gantt chart.

Figure 9 shows the scheduling of the production of three overlapping batches, i.e., the production of one batch is started even though the production of the previous batch is not finished.

The time elapsed between the production of two batches is called the cycle time, which is used to determine the production time required to produce a given number of batches. If there is no overlap, it is equal to the batch time, while, with overlap, it will be the maximum occupation time of the equipment used in manufacturing (*Gutiérrez, 2021*).

There are tasks or operations that can simultaneously occupy more than one piece of equipment in a batch plant, keeping both the equipment being emptied and the equipment being filled occupied.

In the same piece of equipment, the production of a batch cannot start until the previous one has been emptied, from which the concept of production time-limiting equipment arises as the one with the maximum occupancy time per batch.

Thus, for the example in figure 9, from the production of a first batch, a batch can be produced every 2 weeks.

Comparing the productions of the scheduling of figure 8 and figure 9, it can be seen that while without overlapping it takes 31 days to produce 1 single batch, with overlapping, starting from the first batch, a batch could be obtained every 15 days, producing approximately 10 more batches in a year.

With equation 1 it is possible to calculate how many batches would be produced per year (n), knowing that a margin of time must be left, approximately 20 batches are obtained per year.

$$15_{days} \times (n - 1) + 31_{days} = 365_{days/year} \qquad (1)$$

DOWNSTREAM

This phase consists of large-scale production at very large volumes, i.e., at the scale of production required, which will be determined by the production needs (based on the volume of the market for which the drug is intended) and the productivity of the cell line by means of a first filtration stage, followed by 3 chromatography stages and a final concentration stage.

Three chromatography columns (AffC-1000, CatC-1000 and AniC-1000) with a capacity of 1000L each, 4 reactors (R-12500, Raff-3000, Rcat-3000 and Rani-4000) with capacities of 12500L, 3000L, 3000L and 4000L respectively, 5 tanks (T-12500, TW-10000, TlowpH-10000, T-100000 and TmAb-2500) with capacities of 12500L, 10000L, 10000L, 100000L and 2500L respectively, a filter (F-10) of 10L and an ultrafilter (UF-1000) of 1000L.

FILTRATION

CHARGE T-12500	**FILTER F-10**	**SENT TO R-12500**
10640 kg mAbproduction	T-12500	T-12500

AFFINITY CHROMATOGRAPHY

CHARGE TW-10000	**CHARGE TlowpH-10000**	**LOAD AffC-1000**	**SENT TO T-100000**
9000 kg water	8000 kg low Ph tampon	1000 kg TW-10000 10639 kg R-12500	Residue
ELUTE AffC-1000	**SENT TO RAff-3000**		
2500 kg TlowpH-10000	mAb obtained		

CATEXCH CHROMATOGRAPHY

LOAD CatC-1000	**SENT TO Rcat-3000**	**ELUTE CatC-1000**	**SENT TO Rcat-3000**
1000 kg TW-10000 2055 kg RAff-3000	mAb obtained	2000 kg TW-10000	mAb obtained

ANIEXCH CHROMATOGRAPHY

LOAD AniC-1000	**SENT TO Rani-4000**	**ELUTE AniC-1000**	**SENT TO Rani-4000**
1000 kg TW-10000 2800 kg del RCat-3000	mAb obtained	2000 kg TW-10000	mAb obtained

CONCENTRATION

ULTRAFILER UF-1000	**SENT TO T-100000**	**TRANSFER T-2500**
Rani-4000	Bleed	Rani-4000

Finally, 57.9 kg of mAb are obtained for each batch produced. If 20 batches are produced per year, 1158 kg of mAb would be obtained per year.

The complete recipe, for the simulation of the downstream process, as introduced in Aspen, can be found in the annexes.

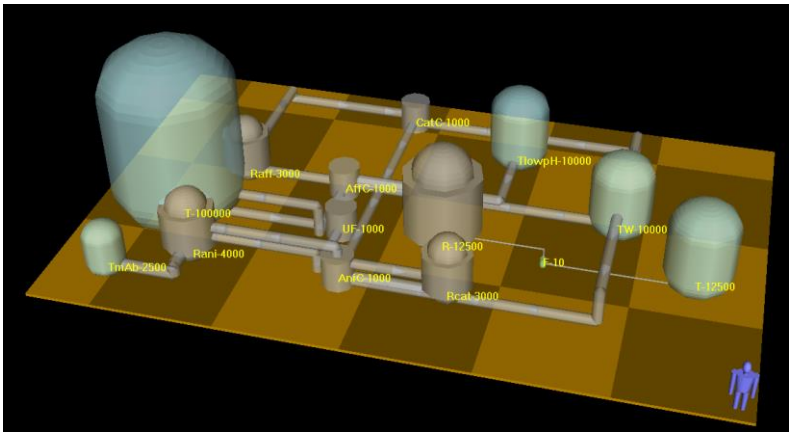


Figure 10: Simulation in Aspen Batch Process Developer of the downstream process.

All suppliers work on a customised design and construction of each individual piece of equipment, which means that the same supplier can offer a variety of different dimensions and conditions for the same piece of equipment.

All the equipment has been selected from local suppliers. As mentioned before, the downstream process consists of fourteen different pieces of equipment, three columns for chromatography, four reactors, five tanks, one filter and one ultrafilter.

A list of the equipment used in the downstream process with their respective local suppliers can be seen in table 3 below *(Aguilar y Salas, 2021; Pfaudler, 2021; Bachiller, 2021; De Dietrich, 2021; Lenntech, 2021).*

Table 3: Downstream equipment suppliers

EQUIPMENTS	SUPPLIERS
Reactors	Aguilar y Salas
Filter	Pfaudler
Tanks	De Dietrich
Chromatography columns	Bachiller
Ultrafilter	Lenntech

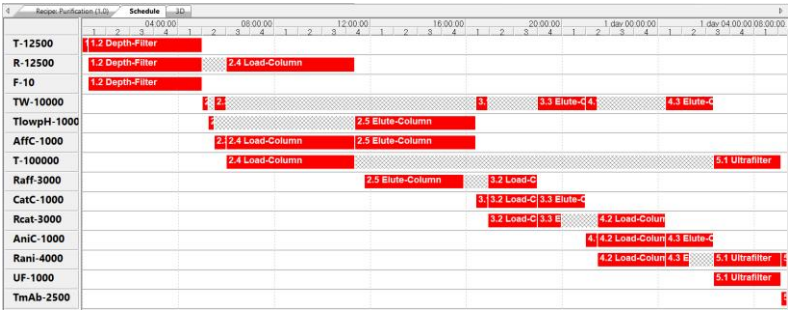


Figure 11: Estimated time of 1 batch expressed in a Gantt chart.

As can be seen in figure 11, the estimated production time for one batch is approximately 29 hours, speaking only of the purification process.

Considering the maintenance operations, it is established that a continuous plant works 8000 hours per year, so, when working in batch mode, we count 6500 hours per year. If the batches are processed consecutively, approximately 250 batches per year could be produced.

Figure 11 shows that the equipment is idle most of the time. Of the 29 hours it takes to manufacture a batch, the T-12500, CatC-1000, AniC-1000 and F-10 units are idle for 24 hours; the TW-10000 unit is idle for 8 hours; the TlowpH-10000, R-12500 and AffC-1000 units are idle for 18 hours; the T-100000 unit is idle for 23 hours; the Rcat-3000, Rani-4000 and Raff-3000 units are idle for 22 hours and the UF-1000 unit is idle for 26 hours.

By overlapping batches, less time is needed to produce a given number of batches.

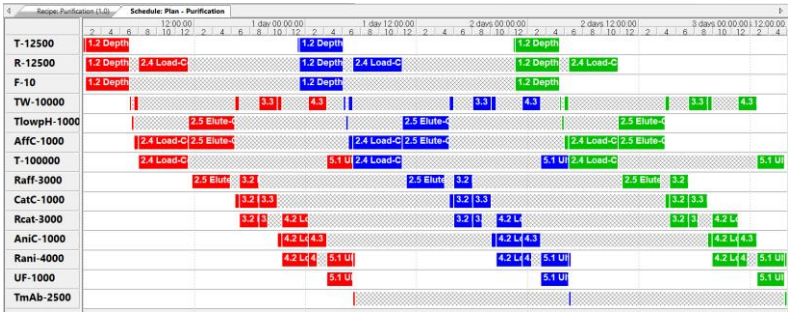


Figure 12: Estimated time of 3 batches with overlaps expressed in a Gantt

Figure 12 shows the scheduling of the production of three overlapping batches, starting the production of one batch, even if the previous one has not been finished.

Thus, for the example in figure 12, from the production of the first batch, a batch can be produced every 22 hours.

Comparing the productions of the programming of figure 11 and figure 12, it can be seen that while without overlapping it takes 29 hours to produce a single batch, with overlapping, starting from the first batch, a batch could be obtained every 22 hours, producing almost 100 more batches in a year.

With equation 2 we can calculate how many batches can be produced per year (n), knowing that we must leave a time margin, approximately 350 batches are obtained per year.

$$22_{hours} \times (n - 1) + 29_{hours} = 6500\ _{hours/year} \qquad (2)$$

It can be seen that production is limited by the upstream process. If more Bevacizumab production is needed, more fermentation tanks of 12500L could be run in parallel. In case no further production is needed, the downstream plant equipment could be partly occupied in the production of other products.

7. CONCLUSIONS

- Due to its wide range of applications for the treatment of different types of cancer, many previous studies and with a personal interest in epithelial ovarian cancer, Bevacizumab has been selected for a study of its preparation process and subsequent design in the plant.
- The legislation and regulations for good manufacturing practices have been studied and it has been determined that during the process, the following must be continuously controlled: the safety and hygiene of the facilities, as well as the safety of the workers, contaminations, validations of the unitary operations, the flow of materials and a good performance of the process.
- A batch production process based on upstream cultivation stages and downstream purification stages has been synthesised.
- The upstream stage consists of 9 fermentations, 6 of them for inoculum and 3 for production, followed by centrifugation.
- The main equipment of the upstream plant is: four reactors in series of increasing size, a 1500L centrifuge and a 12500L tank.
- The downstream stage consists of a first filtration stage, followed by three chromatography stages and a final concentration stage.
- The main equipment of the downstream plant is: three columns for chromatography with a capacity of 1000L each, four reactors and 5 tanks with different capacities, a 10L filter and a 1000L ultrafilter.
- A production plant has been designed with the Aspen Batch Process Developer and by means of scheduling a production capacity of more than 50 kg per batch has been determined, with the possibility of programming approximately 20 batches per year, which are limited by the upstream process. This results in an overall production of around 1000 kg per year.

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ACRONYMS

AEMPS: Spanish Agency for Medicines and Health Products

AEX: Anion exchange chromatography

CEX: Cation exchange chromatography

CHO cells: Chinese hamster ovary cells

CO_2: Carbon dioxide

COGS: Cost of goods sold

DF: Diafiltration

DNA: Deoxyribonucleic acid

EMA: European Medicines Agency

EMEA: Europe, the Middle East and Africa

EU: European Union

Fab: Fixation antigen binding

Fc: Crystallisable fraction

FDA: Federal Food and Drug Administration

GCP: Good clinical practices

GDP: Good distribution practices

GLP: Good laboratory practices

GMP: Good manufacturing practices

GxP: Good process practices

HCP: Host cell protein

hVEGF: Human VEGF

ICH: International Conference on Harmonisation

IND: Investigational New Drug

mAb: Monoclonal Antibody

mRNA: Messenger ribonucleic acid

NGNA: N-Glycolylneuraminic acid

NSO cells: Non-secreting null cells

O_2: Oxygen

Qp: Specific productivity

UF: Ultrafilter

US: United States

VEGF: Vascular endothelial growth factor

WHO: World Health Organisation

APPENDICES

APPENDIX 1: COMPLETE RECIPE OF CELL CULTURE

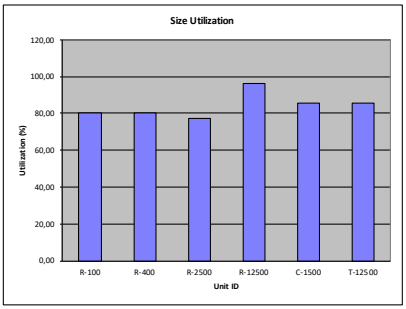
Step Executive Summary

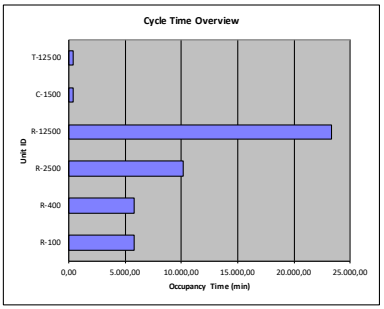
Process (Version):	Culture (1.0)	**Key Input Intermediate:**	CHO
Step (Version):	Fermentation (1.0)	**Key Output Intermediate:**	CHO
Simulation Date:	22/5/21 8:08	**Plan Quantity:**	893,99 kg

Batch Size	Potential Batch Size	Cycle Time	Batch Time	Production Rate	# of Batches	Campaign Time	Campaign Intermediate Production	Total Product Cost	Step Yield
(kg)	(kg)	(min)	(min)	(kg/min)		(min)	(kg)	(USD)	(%)
893,99	931,97	23.376,27	45.276,27	0,04	1	45.276,27	893,99	9.520,00	8.939.886,72

Equipment Capacity

Unit ID	Class	Capacity	Max Capacity	Size Utilized	Unit of Measure	Size Utilization (% Max)	Occupancy Time (min)	Time Utilization (%)
R-100	Reactor	100,00	100,00	80,49	liter	80,49	5.805,00	24,83
R-400	Reactor	400,00	400,00	321,93	liter	80,48	5.820,00	24,90
R-2500	Reactor	2.500,00	2.500,00	1.931,54	liter	77,26	10.176,50	43,53
R-12500	Reactor	12.500,00	12.500,00	11.990,55	liter	95,92	23.376,27	100,00
C-1500	Centrifuge - Disc-Stack	1.500,00	1.500,00	1.279,57	liter	85,30	419,77	1,80
T-12500	Tank	12.500,00	12.500,00	10.709,13	liter	85,67	419,77	1,80





Input Material

Component	Per Batch Amount (kg)	Per Batch Amount (liter)	Campaign Amount (kg)	Campaign Amount (liter)
Insulin	0,00	0,00	0,00	0,00
WHEY	876,92	882,37	876,92	882,37
CHO	0,01	0,01	0,01	0,01
WATER	11.042,93	11.111,66	11.042,93	11.111,66
NITROGEN	39,64	34.623,80	39,64	34.623,80
OXYGEN	12,04	9.203,81	12,04	9.203,81
cysteine	0,15	0,15	0,15	0,15
Copper	0,00	0,00	0,00	0,00
Total Input Material	11.971,69	55.821,81	11.971,69	55.821,81

Output Material

Component	Per Batch Amount (kg)	Campaign Amount (kg)
NITROGEN	39,64	39,64
OXYGEN	12,04	12,04
insulin	0,00	0,00
WHEY	119,20	119,20
CHO	894,00	894,00
WATER	10.847,06	10.847,06
mAB	59,60	59,60
cysteine	0,15	0,15
Copper	0,00	0,00
Total Output Material	11.971,69	11.971,69

Summary

	Per Batch Amount (kg)
Total Input Material	11.971,69
Total Output Material	11.971,69
Discrepancy	0,00

Step Recipe Description

Process (Version): Culture (1.0)
Step (Version): Fermentation (1.0)
Simulation Date: 22/5/21 8:08

Key Input Intermediate: CHO
Key Output Intermediate: CHO
Number of Batches: 1
Plan Quantity: 893,99 kg

Unit ID	Sequence Number	Operation	Operation Liquid+Solid Mass (kg)	Vessel Liquid+Solid Mass (kg)	Operation Liquid+Solid Volume (liter)	Vessel Liquid+Solid Volume (liter)	Operation Time (min)	Vessel Occupancy Time (min)	Batch Time (min)
R-100	1.1.	Charge R-100 with 30 kg of WATER. Charge R-100 with 10 kg of Feed. Charge R-100 with 10 g of CHO.	40,01	40,01	40,26	100,00	15,00	15,00	15,00
	1.2.	Ferment in unit R-100. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 2 day.	0,00	40,01	-0,02	100,00	2.880,00	2.895,00	2.895,00
	1.3.	Charge R-100 with 30 kg of WATER. Charge R-100 with 10 kg of Feed.	40,00	80,01	40,25	100,00	15,00	2.910,00	2.910,00
	1.4.	Ferment in unit R-100. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 2 day.	0,00	80,01	-0,02	100,00	2.880,00	5.790,00	5.790,00
	2.1.	Transfer contents of unit R-100 to R-400. The transfer time is 15 min.	-80,01	0,00	-80,47	100,00	15,00	5.805,00	5.805,00
R-400	2.1.	Transfer contents of unit R-100 to R-400. The transfer time is 15 min.	80,01	80,01	80,47	400,00	15,00	15,00	5.805,00
	2.2.	Charge R-400 with 60 kg of WATER. Charge R-400 with 20 kg of Feed.	80,00	160,01	80,50	400,00	15,00	30,00	5.820,00
	2.3.	Ferment in unit R-400. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 2 day.	0,00	160,01	-0,04	400,00	2.880,00	2.910,00	8.700,00
	2.4.	Charge R-400 with 120 kg of WATER. Charge R-400 with 40 kg of Feed.	160,00	320,01	161,00	400,00	15,00	2.925,00	8.715,00
	2.5.	Ferment in unit R-400. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 2 day.	0,00	320,01	-0,07	400,00	2.880,00	5.805,00	11.595,00
	3.1.	Transfer contents of unit R-400 to R-2500. The transfer time is 15 min.	-320,01	0,00	-321,85	400,00	15,00	5.820,00	11.610,00
R-2500	3.1.	Transfer contents of unit R-400 to R-2500. The transfer time is 15 min.	320,01	320,01	321,85	2.500,00	15,00	15,00	11.610,00
	3.2.	Charge R-2500 with 480 kg of WATER. Charge R-2500 with 120 kg of Feed.	600,00	920,01	603,73	2.500,00	15,00	30,00	11.625,00
	3.3.	Ferment in unit R-2500. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 3 day.	0,00	920,01	-0,28	2.500,00	4.320,00	4.350,00	15.945,00
	3.4.	Charge R-2500 with 800 kg of WATER. Charge R-2500 with 200 kg of Feed.	1.000,00	1.920,01	1.006,22	2.500,00	15,00	4.365,00	15.960,00
	3.5.	Ferment in unit R-2500. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 4 day.	0,00	1.920,01	-0,46	2.500,00	5.760,00	10.125,00	21.720,00
	4.1.	Transfer contents of unit R-2500 to R-12500.	-1.920,01	0,00	-1.931,07	2.500,00	51,50	10.176,50	21.771,50
R-12500	4.1.	Transfer contents of unit R-2500 to R-12500.	1.920,01	1.920,01	1.931,07	12.500,00	51,50	51,50	21.771,50
	4.2.	Charge R-12500 with 1600 kg of WATER. Charge R-12500 with 400 kg of Feed.	2.000,00	3.920,01	2.012,45	12.500,00	15,00	66,50	21.786,50
	4.3.	Ferment in unit R-12500. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 6 day.	0,00	3.920,01	-0,92	12.500,00	8.640,00	8.706,50	30.426,50
	4.4.	Charge R-12500 with 3200 kg of WATER. Charge R-12500 with 800 kg of WHEY	4.000,00	7.920,01	4.024,90	12.500,00	15,00	8.721,50	30.441,50
	4.5.	Ferment in unit R-12500. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 5 day.	0,00	7.920,01	-1,85	12.500,00	7.200,00	15.921,50	37.641,50
	4.6.	Charge R-12500 with 3200 kg of WATER. Charge R-12500 with 800 kg of Feed.	4.000,00	11.920,01	4.024,90	12.500,00	15,00	15.936,50	37.656,50
	4.7.	Ferment in unit R-12500. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 5 day.	0,00	11.920,01	-1,85	12.500,00	7.200,00	23.136,50	44.856,50
	5.1.	Centrifuge the batch from R-12500 in centrifuge C-1500. The transfer rate is 3 Cubic m/h. Transfer 100% of the batch to the centrifuge. The centrifuge speed during slurry charge is 1000 Rev/min. The deliquoring time is 3 h. The speed of rotation during deliquoring is 1000 Rev/min. The centrifuge separates 100% of all solids. The moisture content in the final cake is 30%. The mother liquor, named mAb solution, is sent to T-12500.	-11.920,01	0,00	-11.988,70	12.500,00	239,77	23.376,27	45.096,27
C-1500	5.1.	Centrifuge the batch from R-12500 in centrifuge C-1500. The transfer rate is 3 Cubic m/h. Transfer 100% of the batch to the centrifuge. The centrifuge speed during slurry charge is 1000 Rev/min. The deliquoring time is 3 h. The speed of rotation during deliquoring is 1000 Rev/min. The centrifuge separates 100% of all solids. The moisture content in the final cake is 30%. The mother liquor, named mAb solution, is sent to T-12500.	1.277,13	1.277,13	1.279,57	1.500,00	419,77	419,77	45.276,27
T-12500	5.1.	Centrifuge the batch from R-12500 in centrifuge C-1500. The transfer rate is 3 Cubic m/h. Transfer 100% of the batch to the centrifuge. The centrifuge speed during slurry charge is 1000 Rev/min. The deliquoring time is 3 h. The speed of rotation during deliquoring is 1000 Rev/min. The centrifuge separates 100% of all solids. The moisture content in the final cake is 30%. The mother liquor, named mAb solution, is sent to T-12500.	10.642,88	10.642,88	10.709,13	12.500,00	419,77	419,77	45.276,27

Step Recipe Description

Process (Version): Culture (1.0)
Step (Version): Fermentation (1.0)
Simulation Date: 22/5/21 8:08

Key Input Intermediate: CHO
Key Output Intermediate: CHO
Number of Batches: 1
Plan Quantity: 893,99 kg

Sequence Number	Operation	Charged Material	Average Density (kg/Cubic m)	Average Molecular Weight	Unit ID	Vessel Capacity (liter)	Charge Volume (liter)	Charge Mass (kg)	Vessel Liquid+Solid Volume (liter)	Vessel Volume Occupancy (%)	Operation Time (min)	Batch Time (min)	Volume Equivalent wrt Key Input	Weight Equivalent wrt Key Input	Molar Equivalent wrt Key Input
1.1.	Charge R-100 with 30 kg of WATER. Charge R-100 with 10 kg	WATER	993,81	18,02	R-100	100,00	30,19	30,00	100,00	100,00	15,00	15,00	3.000,00	3.000,00	3.000,00
	of Feed. Charge R-100 with 10 g of CHO.	Insulin (Feed)	993,81	18,02			0,00	0,00					0,00	0,00	0,00
		WHEY (Feed)					0,48	0,48					48,07	48,07	48,07
		WATER (Feed)					9,58	9,52					951,83	951,83	951,83
		cysteine (Feed)					0,00	0,00					0,10	0,10	0,10
		Copper (Feed)					0,00	0,00					0,00	0,00	0,00
		CHO	993,81	18,02			0,01	0,01					1,00	1,00	1,00
1.2.	Ferment in unit R-100. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 2 day.				R-100	100,00			100,00	100,00	2.880,00	2.895,00			
1.3.	Charge R-100 with 30 kg of WATER. Charge R-100 with 10 kg	WATER	993,81	18,02	R-100	100,00	30,19	30,00	100,00	100,00	15,00	2.910,00	3.000,00	3.000,00	3.000,00
	of Feed.	Insulin (Feed)	993,81	18,02			0,00	0,00					0,00	0,00	0,00
		WHEY (Feed)					0,48	0,48					48,07	48,07	48,07
		WATER (Feed)					9,58	9,52					951,83	951,83	951,83
		cysteine (Feed)					0,00	0,00					0,10	0,10	0,10
		Copper (Feed)					0,00	0,00					0,00	0,00	0,00
1.4.	Ferment in unit R-100. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 2 day.				R-100	100,00			100,00	100,00	2.880,00	5.790,00			
2.1.	Transfer contents of unit R-100 to R-400. The transfer time is 15 min.				R-100	100,00			100,00	100,00	15,00	5.805,00			
					R-400	400,00			400,00	100,00	15,00	5.805,00			
2.2.	Charge R-400 with 60 kg of WATER. Charge R-400 with 20 kg	WATER	993,81	18,02	R-400	400,00	60,37	60,00	400,00	100,00	15,00	5.820,00	6.000,00	6.000,00	6.000,00
	of Feed.	Insulin (Feed)	993,81	18,02			0,00	0,00					0,00	0,00	0,00
		WHEY (Feed)					0,97	0,96					96,14	96,14	96,14
		WATER (Feed)					19,16	19,04					1.903,66	1.903,66	1.903,66
		cysteine (Feed)					0,00	0,00					0,19	0,19	0,19
		Copper (Feed)					0,00	0,00					0,00	0,00	0,00
2.3.	Ferment in unit R-400. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 2 day.				R-400	400,00			400,00	100,00	2.880,00	8.700,00			
2.4.	Charge R-400 with 120 kg of WATER. Charge R-400 with 40 kg	WATER	993,81	18,02	R-400	400,00	120,75	120,00	400,00	100,00	15,00	8.715,00	12.000,00	12.000,00	12.000,00
	of Feed.	Insulin (Feed)	993,81	18,02			0,00	0,00					0,00	0,00	0,00
		WHEY (Feed)					1,93	1,92					192,29	192,29	192,29
		WATER (Feed)					38,31	38,07					3.807,32	3.807,32	3.807,32
		cysteine (Feed)					0,00	0,00					0,38	0,38	0,38
		Copper (Feed)					0,00	0,00					0,00	0,00	0,00
2.5.	Ferment in unit R-400. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 2 day.				R-400	400,00			400,00	100,00	2.880,00	11.595,00			
3.1.	Transfer contents of unit R-400 to R-2500. The transfer time is 15 min.				R-400	400,00			400,00	100,00	15,00	11.610,00			
					R-2500	2.500,00			2.500,00	100,00	15,00	11.610,00			
3.2.	Charge R-2500 with 480 kg of WATER. Charge R-2500 with 120 kg of Feed.	WATER	993,81	18,02	R-2500	2.500,00	482,99	480,00	2.500,00	100,00	15,00	11.625,00	48.000,00	48.000,00	48.000,00
		Insulin (Feed)	993,81	18,02			0,00	0,00					0,01	0,01	0,01
		WHEY (Feed)					5,80	5,77					576,87	576,87	576,87
		WATER (Feed)					114,93	114,22					11.421,97	11.421,97	11.421,97
		cysteine (Feed)					0,01	0,01					1,15	1,15	1,15
		Copper (Feed)					0,00	0,00					0,00	0,00	0,00
3.3.	Ferment in unit R-2500. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 3 day.				R-2500	2.500,00			2.500,00	100,00	4.320,00	15.945,00			
3.4.	Charge R-2500 with 800 kg of WATER. Charge R-2500 with 200 kg of Feed.	WATER	993,81	18,02	R-2500	2.500,00	804,98	800,00	2.500,00	100,00	15,00	15.960,00	80.000,00	80.000,00	80.000,00
		Insulin (Feed)	993,81	18,02			0,00	0,00					0,02	0,02	0,02
		WHEY (Feed)					9,67	9,61					961,44	961,44	961,44
		WATER (Feed)					191,55	190,37					19.036,61	19.036,61	19.036,61
		cysteine (Feed)					0,02	0,02					1,92	1,92	1,92
		Copper (Feed)					0,00	0,00					0,00	0,00	0,00
3.5.	Ferment in unit R-2500. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 4 day.				R-2500	2.500,00			2.500,00	100,00	5.760,00	21.720,00			
4.1.	Transfer contents of unit R-2500 to R-12500.				R-2500	2.500,00			2.500,00	100,00	51,50	21.771,50			
					R-12500	12.500,00			12.500,00	100,00	51,50	21.771,50			
4.2.	Charge R-12500 with 1600 kg of WATER. Charge R-12500 with 400 kg of Feed.	WATER	993,81	18,02	R-12500	12.500,00	1.609,96	1.600,00	12.500,00	100,00	15,00	21.786,50	160.000,00	160.000,00	160.000,00
		Insulin (Feed)	993,81	18,02			0,00	0,00					0,04	0,04	0,04
		WHEY (Feed)					19,35	19,23					1.922,89	1.922,89	1.922,89
		WATER (Feed)					383,10	380,73					38.073,22	38.073,22	38.073,22
		cysteine (Feed)					0,04	0,04					3,85	3,85	3,85
		Copper (Feed)					0,00	0,00					0,01	0,01	0,01
4.3.	Ferment in unit R-12500. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 6 day.				R-12500	12.500,00			12.500,00	100,00	8.640,00	30.426,50			
4.4.	Charge R-12500 with 3200 kg of WATER. Charge R-12500 with 800 kg of WHEY.	WATER	993,81	18,02	R-12500	12.500,00	3.219,92	3.200,00	12.500,00	100,00	15,00	30.441,50	320.000,00	320.000,00	320.000,00
		WHEY	993,81	18,02			804,98	800,00					80.000,00	80.000,00	80.000,00
4.5.	Ferment in unit R-12500. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 5 day.				R-12500	12.500,00			12.500,00	100,00	7.200,00	37.641,50			
4.6.	Charge R-12500 with 3200 kg of WATER. Charge R-12500 with 800 kg of Feed.	WATER	993,81	18,02	R-12500	12.500,00	3.219,92	3.200,00	12.500,00	100,00	15,00	37.656,50	320.000,00	320.000,00	320.000,00
		Insulin (Feed)	993,81	18,02			0,00	0,00					0,08	0,08	0,08
		WHEY (Feed)					38,70	38,46					3.845,78	3.845,78	3.845,78
		WATER (Feed)					766,20	761,46					76.146,44	76.146,44	76.146,44
		cysteine (Feed)					0,08	0,08					7,69	7,69	7,69
		Copper (Feed)					0,00	0,00					0,02	0,02	0,02
4.7.	Ferment in unit R-12500. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 5 day.				R-12500	12.500,00			12.500,00	100,00	7.200,00	44.856,50			
5.1.	Centrifuge the batch from R-12500 in centrifuge C-1500. The transfer rate is 3 Cubic m/h. Transfer 100% of the batch to the centrifuge. The centrifuge speed during slurry charge is 1000 Rev/min. The deliquoring time is 3 h. The speed of rotation during deliquoring is 1000 Rev/min. The centrifuge separates 100% of all solids. The moisture content in the final cake is 30%. The mother liquor, named mAb solution, is sent to T-12500.				R-12500	12.500,00			12.500,00	100,00	239,77	45.096,27			
					C-1500	1.500,00			1.500,00	100,00	419,77	45.276,27			
					T-12500	12.500,00			12.500,00	100,00	419,77	45.276,27			

Step Material Balance

Process (Version):	Culture (1.0)	**Key Input Intermediate:**	CHO	
Step (Version):	Fermentation (1.0)	**Key Output Intermediate:**	CHO	
Simulation Date:	22/5/21 8:08	**Number of Batches:**	1	
		Plan Quantity:	893,99	kg

Input Streams

Stream	Component	Per Batch Amount (kg)	Per Batch Amount (liter)	Campaign Amount (kg)	Campaign Amount (liter)	Cost per Unit (USD/kg)	Campaign Cost (USD)
1.1. Charge-1	Total	30,00	30,19	30,00	30,19	1,00	30,00
	WATER	30,00	30,19	30,00	30,19		
1.1. Charge-2	Total	10,00	10,06	10,00	10,06	0,00	0,00
	Insulin	0,00	0,00	0,00	0,00		
	WHEY	0,48	0,48	0,48	0,48		
	WATER	9,52	9,58	9,52	9,58		
	cysteine	0,00	0,00	0,00	0,00		
	Copper	0,00	0,00	0,00	0,00		
1.1. Charge-3	Total	0,01	0,01	0,01	0,01	0,00	0,00
	CHO	0,01	0,01	0,01	0,01		
1.2. Ferment-5	Total	0,00	0,02	0,00	0,02	0,00	0,00
	NITROGEN	0,00	0,01	0,00	0,01		
	OXYGEN	0,00	0,00	0,00	0,00		
1.3. Charge-6	Total	30,00	30,19	30,00	30,19	1,00	30,00
	WATER	30,00	30,19	30,00	30,19		
1.3. Charge-7	Total	10,00	10,06	10,00	10,06	0,00	0,00
	Insulin	0,00	0,00	0,00	0,00		
	WHEY	0,48	0,48	0,48	0,48		
	WATER	9,52	9,58	9,52	9,58		
	cysteine	0,00	0,00	0,00	0,00		
	Copper	0,00	0,00	0,00	0,00		
1.4. Ferment-9	Total	0,00	0,02	0,00	0,02	0,00	0,00
	NITROGEN	0,00	0,01	0,00	0,01		
	OXYGEN	0,00	0,00	0,00	0,00		
2.1. Transfer-11	Total	0,09	80,47	0,09	80,47	0,00	0,00
	NITROGEN	0,07	63,57	0,07	63,57		
	OXYGEN	0,02	16,90	0,02	16,90		
2.2. Charge-13	Total	60,00	60,37	60,00	60,37	1,00	60,00
	WATER	60,00	60,37	60,00	60,37		
2.2. Charge-14	Total	20,00	20,12	20,00	20,12	0,00	0,00
	Insulin	0,00	0,00	0,00	0,00		
	WHEY	0,96	0,97	0,96	0,97		
	WATER	19,04	19,16	19,04	19,16		
	cysteine	0,00	0,00	0,00	0,00		
	Copper	0,00	0,00	0,00	0,00		
2.3. Ferment-16	Total	0,00	0,04	0,00	0,04	0,00	0,00
	NITROGEN	0,00	0,03	0,00	0,03		
	OXYGEN	0,00	0,01	0,00	0,01		
2.4. Charge-17	Total	120,00	120,75	120,00	120,75	1,00	120,00
	WATER	120,00	120,75	120,00	120,75		
2.4. Charge-18	Total	40,00	40,25	40,00	40,25	0,00	0,00
	Insulin	0,00	0,00	0,00	0,00		
	WHEY	1,92	1,93	1,92	1,93		
	WATER	38,07	38,31	38,07	38,31		
	cysteine	0,00	0,00	0,00	0,00		
	Copper	0,00	0,00	0,00	0,00		
2.5. Ferment-20	Total	0,00	0,07	0,00	0,07	0,00	0,00
	NITROGEN	0,00	0,06	0,00	0,06		
	OXYGEN	0,00	0,02	0,00	0,02		
3.1. Transfer-22	Total	0,38	321,85	0,38	321,85	0,00	0,00
	NITROGEN	0,29	254,26	0,29	254,26		
	OXYGEN	0,09	67,59	0,09	67,59		
3.2. Charge-24	Total	480,00	482,99	480,00	482,99	1,00	480,00
	WATER	480,00	482,99	480,00	482,99		
3.2. Charge-25	Total	120,00	120,75	120,00	120,75	0,00	0,00
	Insulin	0,00	0,00	0,00	0,00		
	WHEY	5,77	5,80	5,77	5,80		
	WATER	114,22	114,93	114,22	114,93		
	cysteine	0,01	0,01	0,01	0,01		
	Copper	0,00	0,00	0,00	0,00		
3.3. Ferment-27	Total	0,00	0,28	0,00	0,28	0,00	0,00
	NITROGEN	0,00	0,22	0,00	0,22		
	OXYGEN	0,00	0,06	0,00	0,06		
3.4. Charge-28	Total	800,00	804,98	800,00	804,98	1,00	800,00
	WATER	800,00	804,98	800,00	804,98		
3.4. Charge-29	Total	200,00	201,24	200,00	201,24	0,00	0,00
	Insulin	0,00	0,00	0,00	0,00		
	WHEY	9,61	9,67	9,61	9,67		
	WATER	190,37	191,55	190,37	191,55		
	cysteine	0,02	0,02	0,02	0,02		
	Copper	0,00	0,00	0,00	0,00		
3.5. Ferment-31	Total	0,00	0,46	0,00	0,46	0,00	0,00
	NITROGEN	0,00	0,36	0,00	0,36		
	OXYGEN	0,00	0,10	0,00	0,10		
4.1. Transfer-33	Total	2,28	1.931,07	2,28	1.931,07	0,00	0,00
	NITROGEN	1,75	1.525,55	1,75	1.525,55		
	OXYGEN	0,53	405,53	0,53	405,53		
4.2. Charge-35	Total	1.600,00	1.609,96	1.600,00	1.609,96	1,00	1.600,00
	WATER	1.600,00	1.609,96	1.600,00	1.609,96		
4.2. Charge-36	Total	400,00	402,49	400,00	402,49	0,00	0,00
	Insulin	0,00	0,00	0,00	0,00		
	WHEY	19,23	19,35	19,23	19,35		
	WATER	380,73	383,10	380,73	383,10		
	cysteine	0,04	0,04	0,04	0,04		
	Copper	0,00	0,00	0,00	0,00		
4.3. Ferment-38	Total	0,00	0,92	0,00	0,92	0,00	0,00
	NITROGEN	0,00	0,73	0,00	0,73		
	OXYGEN	0,00	0,19	0,00	0,19		
4.4. Charge-39	Total	3.200,00	3.219,92	3.200,00	3.219,92	1,00	3.200,00
	WATER	3.200,00	3.219,92	3.200,00	3.219,92		
4.4. Charge-40	Total	800,00	804,98	800,00	804,98	0,00	0,00
	WHEY	800,00	804,98	800,00	804,98		
4.5. Ferment-42	Total	0,00	1,85	0,00	1,85	0,00	0,00
	NITROGEN	0,00	1,46	0,00	1,46		
	OXYGEN	0,00	0,39	0,00	0,39		
4.6. Charge-43	Total	3.200,00	3.219,92	3.200,00	3.219,92	1,00	3.200,00
	WATER	3.200,00	3.219,92	3.200,00	3.219,92		
4.6. Charge-44	Total	800,00	804,98	800,00	804,98	0,00	0,00
	Insulin	0,00	0,00	0,00	0,00		
	WHEY	38,46	38,70	38,46	38,70		
	WATER	761,46	766,20	761,46	766,20		
	cysteine	0,08	0,08	0,08	0,08		
	Copper	0,00	0,00	0,00	0,00		
4.7. Ferment-46	Total	0,00	1,85	0,00	1,85	0,00	0,00
	NITROGEN	0,00	1,46	0,00	1,46		
	OXYGEN	0,00	0,39	0,00	0,39		
5.1. Centrifuge-49	Total	14,14	11.988,70	14,14	11.988,70	0,00	0,00
	NITROGEN	10,84	9.471,07	10,84	9.471,07		
	OXYGEN	3,29	2.517,63	3,29	2.517,63		
Total Input Material		11.936,90	26.321,81	11.936,90	26.321,81		9.520,00

Output Streams

Stream	Component	Per Batch Amount (kg)	Per Batch Amount (liter)	Campaign Amount (kg)	Campaign Amount (liter)	Cost per Unit (USD/kg)	Campaign Cost (USD)
1.1. Charge-4	Total	0,05	40,26	0,05	40,26	0,00	0,00
	NITROGEN	0,04	31,80	0,04	31,80		
	OXYGEN	0,01	8,45	0,01	8,45		
1.3. Charge-8	Total	0,05	40,25	0,05	40,25	0,00	0,00
	NITROGEN	0,04	31,80	0,04	31,80		
	OXYGEN	0,01	8,45	0,01	8,45		
2.1. Transfer-12	Total	0,09	80,47	0,09	80,47	0,00	0,00
	NITROGEN	0,07	63,57	0,07	63,57		
	OXYGEN	0,02	16,90	0,02	16,90		
2.2. Charge-15	Total	0,09	80,50	0,09	80,50	0,00	0,00
	NITROGEN	0,07	63,59	0,07	63,59		
	OXYGEN	0,02	16,90	0,02	16,90		
2.4. Charge-19	Total	0,19	161,00	0,19	161,00	0,00	0,00
	NITROGEN	0,15	127,19	0,15	127,19		
	OXYGEN	0,04	33,81	0,04	33,81		
3.1. Transfer-23	Total	0,38	321,85	0,38	321,85	0,00	0,00
	NITROGEN	0,29	254,26	0,29	254,26		
	OXYGEN	0,09	67,59	0,09	67,59		
3.2. Charge-26	Total	0,71	603,73	0,71	603,73	0,00	0,00
	NITROGEN	0,55	476,95	0,55	476,95		
	OXYGEN	0,17	126,78	0,17	126,78		
3.4. Charge-30	Total	1,19	1.006,22	1,19	1.006,22	0,00	0,00
	NITROGEN	0,91	794,92	0,91	794,92		
	OXYGEN	0,28	211,31	0,28	211,31		
4.1. Transfer-34	Total	2,28	1.931,07	2,28	1.931,07	0,00	0,00
	NITROGEN	1,75	1.525,55	1,75	1.525,55		
	OXYGEN	0,53	405,53	0,53	405,53		
4.2. Charge-37	Total	2,37	2.012,45	2,37	2.012,45	0,00	0,00
	NITROGEN	1,82	1.589,83	1,82	1.589,83		
	OXYGEN	0,55	422,61	0,55	422,61		
4.4. Charge-41	Total	4,75	4.024,90	4,75	4.024,90	0,00	0,00
	NITROGEN	3,64	3.179,67	3,64	3.179,67		
	OXYGEN	1,11	845,23	1,11	845,23		
4.6. Charge-45	Total	4,75	4.024,90	4,75	4.024,90	0,00	0,00
	NITROGEN	3,64	3.179,67	3,64	3.179,67		
	OXYGEN	1,11	845,23	1,11	845,23		
5.1. Centrifuge-50	Total	1,51	1.279,57	1,51	1.279,57	0,00	0,00
	NITROGEN	1,16	1.010,86	1,16	1.010,86		
	OXYGEN	0,35	268,71	0,35	268,71		
5.1. Centrifuge-51	Total	12,63	10.709,13	12,63	10.709,13	0,00	0,00
	NITROGEN	9,69	8.460,21	9,69	8.460,21		
	OXYGEN	2,94	2.248,92	2,94	2.248,92		
Total Output Material		31,03	26.316,31	31,03	26.316,31		0,00

Initial Equipment Contents

Unit ID	Component	Per Batch Amount (kg)
R-100	Total	0,12
	NITROGEN	0,09
	OXYGEN	0,03
R-400	Total	0,47
	NITROGEN	0,36
	OXYGEN	0,11
R-2500	Total	2,95
	NITROGEN	2,26
	OXYGEN	0,69
R-12500	Total	14,74
	NITROGEN	11,31
	OXYGEN	3,43
C-1500	Total	1,77
	NITROGEN	1,36
	OXYGEN	0,41
T-12500	Total	14,74
	NITROGEN	11,31
	OXYGEN	3,43
Total Initial Contents		34,78

Final Equipment Contents

Unit ID	Component	Per Batch Amount (kg)
R-100	Total	0,12
	NITROGEN	0,09
	OXYGEN	0,03
R-400	Total	0,47
	NITROGEN	0,36
	OXYGEN	0,11
R-2500	Total	2,95
	NITROGEN	2,26
	OXYGEN	0,69
R-12500	Total	14,74
	NITROGEN	11,31
	OXYGEN	3,43
C-1500	Total	1.277,39
	Insulin	0,00
	WHEY	4,14
	CHO	893,99
	WATER	376,92
	NITROGEN	0,20
	OXYGEN	0,06
	mAB	2,07
	cysteine	0,01
	Copper	0,00
T-12500	Total	10.645,00
	Insulin	0,00
	WHEY	115,06
	CHO	0,01
	WATER	10.470,14
	NITROGEN	1,62
	OXYGEN	0,49
	mAB	57,53
	cysteine	0,15
	Copper	0,00
Total Final Contents		11.940,66

Summary

	Per Batch Amount (kg)
Total Input Streams	11.936,90
Total Output Streams	31,03
Total Initial Contents	34,78
Total Final Contents	11.940,66
Discrepancy	0,00

Step Recipe Sequence Diagram

Process (Version):	Culture (1.0)	**Key Input Intermediate:**	CHO
Step (Version):	Fermentation (1.0)	**Key Output Intermediate:**	CHO
Simulation Date:	##########	**Number of Batches:**	1
		Plan Quantity:	692,99

Input Streams	From	Mass (kg)	Volume (liter)	Temp (C)	Start Time (min)	End Time (min)	Operation Time (min)	Sequence Number	R-100	Vessel Liq+Sol Mass (kg)	Vessel Liq+Sol Volume (liter)	Vessel Temp (C)	Vessel Pressure (kPa)	Mass (kg)	Volume (liter)	Temp (C)	To	Output Streams
WATER		30,00	30,19	25,00	0,00	15,00	15,00	1.1.	Charge R-100 with 30 kg of WATER. Charge R-100 with 10 kg of Feed. Charge R-100 with 10 g of CHO.	40,01	100,00	25,00	101,33					
Feed		10,00	10,06	25,00														
CHO		0,01	0,01	25,00														
					15,00	2.895,00	2.880,00	1.2.	Ferment in unit R-100. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 2 day.	40,01	100,00	25,00	101,33					
WATER		30,00	30,19	25,00	2.895,00	2.910,00	15,00	1.3.	Charge R-100 with 30 kg of WATER. Charge R-100 with 10 kg of Feed.	80,01	100,00	25,00	101,33	0,05	40,25	25,00		1.3. Charge-8
Feed		10,00	10,06	25,00														
					2.910,00	5.790,00	2.880,00	1.4.	Ferment in unit R-100. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 2 day.	80,01	100,00	25,00	101,33					
					5.790,00	5.805,00	15,00	2.1.	Transfer contents of unit R-100 to R-400. The transfer time is 15 min.	0,00	100,00	25,00	101,32	80,01	80,47	25,00	R-400	2.1. Transfer-10

Input Streams	From	Mass (kg)	Volume (liter)	Temp (C)	Start Time (min)	End Time (min)	Operation Time (min)	Sequence Number	R-400	Vessel Liq+Sol Mass (kg)	Vessel Liq+Sol Volume (liter)	Vessel Temp (C)	Vessel Pressure (kPa)	Mass (kg)	Volume (liter)	Temp (C)	To	Output Streams
2.1. Transfer-10	R-100	80,01	80,47	25,00	5.790,00	5.805,00	15,00	2.1.	Transfer contents of unit R-100 to R-400. The transfer time is 15 min.	80,01	400,00	25,00	101,33					
WATER		60,00	60,37	25,00	5.805,00	5.820,00	15,00	2.2.	Charge R-400 with 60 kg of WATER. Charge R-400 with 20 kg of Feed.	160,01	400,00	25,00	101,33					
Feed		20,00	20,12	25,00														
					5.820,00	8.700,00	2.880,00	2.3.	Ferment in unit R-400. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 2 day.	160,01	400,00	25,00	101,33					
WATER		120,00	120,75	25,00	8.700,00	8.715,00	15,00	2.4.	Charge R-400 with 120 kg of WATER. Charge R-400 with 40 kg of Feed.	320,01	400,00	25,00	101,33	0,19	161,00	25,00		2.4. Charge-19
Feed		40,00	40,25	25,00														
					8.715,00	11.595,00	2.880,00	2.5.	Ferment in unit R-400. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 2 day.	320,01	400,00	25,00	101,33					
					11.595,00	11.610,00	15,00	3.1.	Transfer contents of unit R-400 to R-2500. The transfer time is 15 min.	0,00	400,00	25,00	101,32	320,01	321,85	25,00	R-2500	3.1. Transfer-21

Input Streams	From	Mass (kg)	Volume (liter)	Temp (C)	Start Time (min)	End Time (min)	Operation Time (min)	Sequence Number	R-2500	Vessel Liq+Sol Mass (kg)	Vessel Liq+Sol Volume (liter)	Vessel Temp (C)	Vessel Pressure (kPa)	Mass (kg)	Volume (liter)	Temp (C)	To	Output Streams
3.1. Transfer-21	R-400	320,01	321,85	25,00	11.595,00	11.610,00	15,00	3.1.	Transfer contents of unit R-400 to R-2500. The transfer time is 15 min.	320,01	2.500,00	25,00	101,33					
WATER		480,00	482,99	25,00	11.610,00	11.625,00	15,00	3.2.	Charge R-2500 with 480 kg of WATER. Charge R-2500 with 120 kg of Feed.	920,01	2.500,00	25,00	101,33					
Feed		120,00	120,75	25,00														
					11.625,00	15.945,00	4.320,00	3.3.	Ferment in unit R-2500. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 3 day.	920,01	2.500,00	25,00	101,33					
WATER		800,00	804,98	25,00	15.945,00	15.960,00	15,00	3.4.	Charge R-2500 with 800 kg of WATER. Charge R-2500 with 200 kg of Feed.	1.920,01	2.500,00	25,00	101,33	1,19	1.006,22	25,00		3.4. Charge-30
Feed		200,00	201,24	25,00														
					15.960,00	21.720,00	5.760,00	3.5.	Ferment in unit R-2500. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 4 day.	1.920,01	2.500,00	25,00	101,33					
					21.720,00	21.771,50	51,50	4.1.	Transfer contents of unit R-2500 to R-12500.	0,00	2.500,00	25,00	101,33	1.920,01	1.931,07	25,00	R-12500	4.1. Transfer-32

Input Streams	From	Mass (kg)	Volume (liter)	Temp (C)	Start Time (min)	End Time (min)	Operation Time (min)	Sequence Number	R-12500	Vessel Liq+Sol Mass (kg)	Vessel Liq+Sol Volume (liter)	Vessel Temp (C)	Vessel Pressure (kPa)	Mass (kg)	Volume (liter)	Temp (C)	To	Output Streams
4.1. Transfer-32	R-2500	1.920,01	1.931,07	25,00	21.720,00	21.771,50	51,50	4.1.	Transfer contents of unit R-2500 to R-12500.	1.920,01	12.500,00	25,00	101,33					
WATER		1.600,00	1.609,96	25,00	21.771,50	21.786,50	15,00	4.2.	Charge R-12500 with 1600 kg of WATER. Charge R-12500 with 400 kg of Feed.	3.920,01	12.500,00	25,00	101,33					
Feed		400,00	402,49	25,00														
					21.786,50	30.426,50	8.640,00	4.3.	Ferment in unit R-12500. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 6 day.	3.920,01	12.500,00	25,00	101,33					
WATER		3.200,00	3.219,92	25,00	30.426,50	30.441,50	15,00	4.4.	Charge R-12500 with 3200 kg of WATER. Charge R-12500 with 800 kg of WHEY.	7.920,01	12.500,00	25,00	101,33	4,75	4.024,90	25,00		4.4. Charge-41
WHEY		800,00	804,98	25,00														
					30.441,50	37.641,50	7.200,00	4.5.	Ferment in unit R-12500. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 5 day.	7.920,01	12.500,00	25,00	101,33					
WATER		3.200,00	3.219,92	25,00	37.641,50	37.656,50	15,00	4.6.	Charge R-12500 with 3200 kg of WATER. Charge R-12500 with 800 kg of Feed.	11.920,01	12.500,00	25,00	101,33	4,75	4.024,90	25,00		4.6. Charge-45
Feed		800,00	804,98	25,00														
					37.656,50	44.856,50	7.200,00	4.7.	Ferment in unit R-12500. The yield of CHO in the Solid phase is 0,075, of mAB in the Liquid phase is 0,005, of WATER in the Liquid phase is 0,91 and of WHEY in the Liquid phase is 0,01. The fermentation time is 5 day.	11.920,01	12.500,00	25,00	101,33					
					44.856,50	45.096,27	239,77	5.1.	Centrifuge the batch from R-12500 in centrifuge C-1500. The transfer rate is 3 Cubic m/h. Transfer 100% of the batch to the centrifuge. The centrifuge speed during slurry charge is 1000 Rev/min. The deliquoring time is 3 h. The speed of rotation during deliquoring is 1000 Rev/min. The centrifuge separates 100% of all solids. The moisture content in the final cake is 30%. The mother liquor, named mAb solution, is sent to T-12500.	0,00	12.500,00	25,00	101,33	11.920,01	11.988,70	25,00	C-1500	5.1. Centrifuge-47

Input Streams	From	Mass (kg)	Volume (liter)	Temp (C)	Start Time (min)	End Time (min)	Operation Time (min)	Sequence Number	C-1500	Vessel Liq+Sol Mass (kg)	Vessel Liq+Sol Volume (liter)	Vessel Temp (C)	Vessel Pressure (kPa)	Mass (kg)	Volume (liter)	Temp (C)	To	Output Streams
5.1. Centrifuge-47	R-12500	11.920,01	11.988,70	25,00	44.856,50	45.276,27	419,77	5.1.	Centrifuge the batch from R-12500 in centrifuge C-1500. The transfer rate is 3 Cubic m/h. Transfer 100% of the batch to the centrifuge. The centrifuge speed during slurry charge is 1000 Rev/min. The deliquoring time is 3 h. The speed of rotation during deliquoring is 1000 Rev/min. The centrifuge separates 100% of all solids. The moisture content in the final cake is 30%. The mother liquor, named mAb solution, is sent to T-12500.	1.277,13	1.500,00	25,00	101,33	10.642,88	10.709,13	25,00	T-12500	mAb solution

Input Streams	From	Mass (kg)	Volume (liter)	Temp (C)	Start Time (min)	End Time (min)	Operation Time (min)	Sequence Number	T-12500	Vessel Liq+Sol Mass (kg)	Vessel Liq+Sol Volume (liter)	Vessel Temp (C)	Vessel Pressure (kPa)	Mass (kg)	Volume (liter)	Temp (C)	To	Output Streams
mAb solution	C-1500	10.642,88	10.709,13	25,00	44.856,50	45.276,27	419,77	5.1.	Centrifuge the batch from R-12500 in centrifuge C-1500. The transfer rate is 3 Cubic m/h. Transfer 100% of the batch to the centrifuge. The centrifuge speed during slurry charge is 1000 Rev/min. The deliquoring time is 3 h. The speed of rotation during deliquoring is 1000 Rev/min. The centrifuge separates 100% of all solids. The moisture content in the final cake is 30%. The mother liquor, named mAb solution, is sent to T-12500.	10.642,88	12.500,00	25,00	101,33					

APPENDIX 2: COMPLETE RECIPE OF PURIFICATION

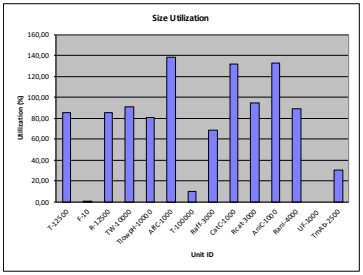
Step Executive Summary

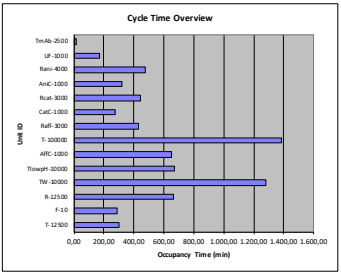
Process (Version):	Purification (1.0)	**Key Input Intermediate:**	mAb		
Step (Version):	Purification (1.0)	**Key Output Intermediate:**	mAb		
Simulation Date:	22/5/21 9:19	**Plan Quantity:**	55,38	kg	

Batch Size	Potential	Batch Size	Cycle Time	Batch Time	Production Rate	# of Batches	Campaign Time	Campaign Intermediate Production	Total Product Cost	Step Yield
(kg)		(kg)	(min)	(min)	(kg/min)		(min)	(kg)	(USD)	(%)
55,38		39,98	1.386,80	1.762,49	0,04	1	1.762,49	55,38	9.000,00	95,66

Equipment Capacity

Unit ID	Class	Capacity	Max Capacity	Size Utilized	Unit of Measure	Size Utilization (% Max)	Occupancy Time (min)	Time Utilization (%)
T-12500	Tank	12.500,00	12.500,00	10.706,23	liter	85,65	300,50	21,67
F-10	Filter - Depth	10,00	10,00	0,01	liter	0,10	285,50	20,59
R-12500	Reactor	12.500,00	12.500,00	10.706,22	liter	85,65	666,84	48,08
TW-10000	Tank	10.000,00	10.000,00	9.056,02	liter	90,56	1.278,30	92,18
TlowpH-10000	Tank	10.000,00	10.000,00	8.051,78	liter	80,52	668,28	48,19
AffC-1000	Column - Chromatography	1.000,00	950,00	1.315,84	liter	138,51	653,28	47,11
T-100000	Tank	100.000,00	100.000,00	10.395,61	liter	10,40	1.386,80	100,00
Raff-3000	Reactor	3.000,00	3.000,00	2.071,04	liter	69,03	432,07	31,16
CatC-1000	Column - Chromatography	1.000,00	950,00	1.252,44	liter	131,84	275,02	19,83
Rcat-3000	Reactor	3.000,00	3.000,00	2.828,81	liter	94,29	444,08	32,02
AniC-1000	Column - Chromatography	1.000,00	950,00	1.261,93	liter	132,84	320,00	23,07
Rani-4000	Reactor	4.000,00	4.000,00	3.569,36	liter	89,23	473,50	34,14
UF-1000	Ultrafilter	1.000,00	1.000,00	0,00	liter	0,00	168,69	12,16
TmAb-2500	Tank	2.500,00	2.500,00	757,94	liter	30,32	15,00	1,08





Input Material

Component	Per Batch	Amount (kg)	Per Batch Amount (liter)	Campaign Amount (kg)	Campaign Amount (liter)
Insulin		0,00	0,00	0,00	0,00
WHEY		115,79	116,51	115,79	116,51
CHO		0,01	0,01	0,01	0,01
HYDROGEN-CHLORIDE		7,99	10,02	7,99	10,02
WATER		27.458,15	27.629,06	27.458,15	27.629,06
NITROGEN		181,83	158.818,71	181,83	158.818,71
OXYGEN		55,22	42.217,69	55,22	42.217,69
mAb		57,90	58,26	57,90	58,26
cysteine		0,15	0,15	0,15	0,15
Copper		0,00	0,00	0,00	0,00
Total Input Material		27.877,05	228.850,42	27.877,05	228.850,42

Output Material

Component	Per Batch	Amount (kg)	Campaign Amount (kg)
Insulin		0,00	0,00
WHEY		115,79	115,79
HYDROGEN-CHLORIDE		7,99	7,99
WATER		27.458,15	27.458,15
NITROGEN		181,83	181,83
OXYGEN		55,22	55,22
mAb		57,90	57,90
cysteine		0,15	0,15
Copper		0,00	0,00
CHO		0,01	0,01
Total Output Material		27.877,05	27.877,05

Summary

	Per Batch Amount (kg)
Total Input Material	27.877,05
Total Output Material	27.877,05
Discrepancy	0,00

Step Recipe Description

Process (Version): Purification (1.0)
Step (Version): Purification (1.0)
Simulation Date: 22/5/21 9:19

Key Input Intermediate: mAb
Key Output Intermediate: mAb
Number of Batches: 1
Plan Quantity: 55,38 kg

Unit ID	Sequence Number	Operation	Operation Liquid+Solid Mass (kg)	Vessel Liquid+Solid Mass (kg)	Operation Liquid+Solid Volume (liter)	Vessel Liquid+Solid Volume (liter)	Operation Time (min)	Vessel Occupancy Time (min)	Batch Time (min)
T-12500	1.1.	Charge T-12500 with 10640 kg of mAbproduction. The charge time is 15 min.	10.640,00	10.640,00	10.706,23	12.500,00	15,00	15,00	15,00
	1.2.	Filter the contents of T-12500 in depth filter F-10. The transfer rate is 2,25 Cubic m/h. The filtrate, named mAbfiltrate, is sent to R-12500.	-10.640,00	0,00	-10.706,23	12.500,00	285,50	300,50	300,50
F-10	1.2.	Filter the contents of T-12500 in depth filter F-10. The transfer rate is 2,25 Cubic m/h. The filtrate, named mAbfiltrate, is sent to R-12500.	0,01	0,01	0,01	10,00	285,50	285,50	300,50
R-12500	1.2.	Filter the contents of T-12500 in depth filter F-10. The transfer rate is 2,25 Cubic m/h. The filtrate, named mAbfiltrate, is sent to R-12500.	10.639,99	10.639,99	10.706,22	12.500,00	285,50	285,50	300,50
	2.4.	Load the column AffC-1000. Use 10639 kg of the material in R-12500. The transfer rate is 2 Bed Volume/h. The resin retains 98% of mAb, 5% of cysteine, 5% of Insulin, 5% of Copper, 5% of WHEY and 0,01% of WATER. Displaced Liquid Stream: The displaced liquid, named Residue, is sent to T-100000.	-10.639,01	0,98	-10.705,23	12.500,00	321,16	666,84	681,84
TW-10000	2.1.	Charge TW-10000 with 9000 kg of WATER.	9.000,00	9.000,00	9.056,02	10.000,00	15,00	15,00	315,50
	2.3.	Load the column AffC-1000. Use 1000 kg of the material in TW-10000. The transfer rate is 2 Bed Volume/h. The resin retains 30% of WATER.	-1.000,00	8.000,00	-1.006,22	10.000,00	30,19	60,19	360,69
	3.1.	Load the column CatC-1000. Use 1000 kg of the material in TW-10000. The transfer rate is 2 Bed Volume/h. The resin retains 30% of WATER.	-1.000,00	7.000,00	-1.006,22	10.000,00	30,19	713,47	1.013,97
	3.3.	Elute the column CatC-1000 with 2000 kg of the material in TW-10000. Collect 1000 kg at 0 kg into the elution. The cut, named mAbcat(2), is sent to Rcat-3000. The solvent removes 100% of mAb, 10% of WHEY, 10% of cysteine and 10% of Insulin.	-2.000,00	5.000,00	-2.012,45	10.000,00	120,75	958,30	1.258,80
	4.1.	Load the column AniC-1000. Use 1000 kg of the material in TW-10000. The transfer rate is 2 Bed Volume/h. The resin retains 30% of WATER.	-1.000,00	4.000,00	-1.006,22	10.000,00	30,19	988,49	1.288,99
	4.3.	Elute the column AniC-1000 with 2000 kg of the material in TW-10000. Collect 1000 kg at 0 kg into the elution. The cut, named mAbani(2), is sent to Rani-4000. The solvent removes 100% of mAb, 10% of WHEY, 10% of cysteine and 10% of Insulin.	-2.000,00	2.000,00	-2.012,45	10.000,00	120,75	1.278,30	1.578,80
TlowpH-10000	2.2.	Charge TlowpH-10000 with 8000 kg of Low pH tampon.	8.000,00	8.000,00	8.051,78	10.000,00	15,00	15,00	330,50
	2.5.	Elute the column AffC-1000 with 2500 kg of the material in TlowpH-10000. The elution rate is 0,5 Bed Volume/h. Collect 2000 kg at 200 kg into the elution. The cut, named mAbAff, is sent to Raff-3000. The solvent removes 98% of mAb, 60% of cysteine, 60% of Insulin, 95% of Copper and 40% of WHEY. Unspecified components remain in the column.	-2.500,00	5.500,00	-2.516,18	10.000,00	301,94	668,28	983,78
AffC-1000	2.3.	Load the column AffC-1000. Use 1000 kg of the material in TW-10000. The transfer rate is 2 Bed Volume/h. The resin retains 30% of WATER.	1.000,00	1.000,00	1.006,22	1.006,22	30,19	30,19	360,69
	2.4.	Load the column AffC-1000. Use 10639 kg of the material in R-12500. The transfer rate is 2 Bed Volume/h. The resin retains 98% of mAb, 5% of cysteine, 5% of Insulin, 5% of Copper, 5% of WHEY and 0,01% of WATER. Displaced Liquid Stream: The displaced liquid, named Residue, is sent to T-100000.	307,70	1.307,70	309,61	1.315,84	321,16	351,34	681,84
	2.5.	Elute the column AffC-1000 with 2500 kg of the material in TlowpH-10000. The elution rate is 0,5 Bed Volume/h. Collect 2000 kg at 200 kg into the elution. The cut, named mAbAff, is sent to Raff-3000. The solvent removes 98% of mAb, 60% of cysteine, 60% of Insulin, 95% of Copper and 40% of WHEY. Unspecified components remain in the column.	-58,15	1.249,55	-58,28	1.257,56	301,94	653,28	983,78

T-100000	2.4.	Load the column ARC-1000. Use 10630 kg of the material in R-12500. The transfer rate is 2 Bed Volume/h. The resin retains 98% of mAb, 5% of cysteine, 5% of Insulin, 5% of Copper, 5% of WHEY and 0,01% of WATER. Displaced Liquid Stream: The displaced liquid, named Residue, is sent to T-100000.	10.331,30	10.331,30	10.395,61	100.000,00	321,16	321,16	681,84
	5.1.	Ultrafilter the contents of Rani-4000 in UF-1000. The mode of operation is Feed and Bleed. Recycle 100% of the retentate. The bleed is sent to T-100000. Separation is: 100% of mAb in Liquid goes to the Retentate and 20% of WATER in Liquid goes to the Retentate. 100% of each unspecified component goes to Permeate.	0,00	10.331,30	0,00	100.000,00	168,69	1.386,80	1.747,49
Raff-3000	2.5.	Elute the column ARC-1000 with 2500 kg of the material in TlowpH-10000. The elution rate is 0,5 Bed Volume/h. Collect 2000 kg at 200 kg into the elution. The cut, named mAbAff, is sent to Raff-3000. The solvent removes 98% of mAb, 60% of cysteine, 60% of Insulin, 95% of Copper and 40% of WHEY. Unspecified components remain in the column.	2.057,92	2.057,92	2.071,04	3.000,00	248,52	248,52	954,52
	3.2.	Load the column CatC-1000. Use 2055 kg of the material in Raff-3000. The transfer rate is 1 Bed Volume/h. The resin retains 5% of Copper, 95% of cysteine, 30% of Insulin and 1% of mAb. Displaced Liquid Stream: The displaced liquid, named mAbcat, is sent to Rcat-3000.	-2.054,99	2,93	-2.068,09	3.000,00	124,09	432,07	1.138,06
CatC-1000	3.1.	Load the column CatC-1000. Use 1000 kg of the material in TW-10000. The transfer rate is 2 Bed Volume/h. The resin retains 30% of WATER.	1.000,00	1.000,00	1.006,22	1.006,22	30,19	30,19	1.013,97
	3.2.	Load the column CatC-1000. Use 2055 kg of the material in Raff-3000. The transfer rate is 1 Bed Volume/h. The resin retains 5% of Copper, 95% of cysteine, 30% of Insulin and 1% of mAb. Displaced Liquid Stream: The displaced liquid, named mAbcat, is sent to Rcat-3000.	244,55	1.244,55	246,22	1.252,44	124,09	154,27	1.138,06
	3.3.	Elute the column CatC-1000 with 2000 kg of the material in TW-10000. Collect 1000 kg at 0 kg into the elution. The cut, named mAbcat(2), is sent to Rcat-3000. The solvent removes 100% of mAb, 10% of WHEY, 10% of cysteine and 10% of Insulin.	-300,43	944,12	-302,44	1.000,00	120,75	275,02	1.258,80
Rcat-3000	3.2.	Load the column CatC-1000. Use 2055 kg of the material in Raff-3000. The transfer rate is 1 Bed Volume/h. The resin retains 5% of Copper, 95% of cysteine, 30% of Insulin and 1% of mAb. Displaced Liquid Stream: The displaced liquid, named mAbcat, is sent to Rcat-3000.	1.810,45	1.810,45	1.821,89	3.000,00	124,09	124,09	1.138,06
	3.3.	Elute the column CatC-1000 with 2000 kg of the material in TW-10000. Collect 1000 kg at 0 kg into the elution. The cut, named mAbcat(2), is sent to Rcat-3000. The solvent removes 100% of mAb, 10% of WHEY, 10% of cysteine and 10% of Insulin.	1.000,56	2.811,01	1.006,93	3.000,00	60,42	184,50	1.198,47
	4.2.	Load the column AnIC-1000. Use 2800 kg of the material in Rcat-3000. The resin retains 100% of Copper, 100% of cysteine, 100% of Insulin and 100% of WHEY. Displaced Liquid Stream: The displaced liquid, named mAbani, is sent to Rani-4000.	-2.800,00	11,01	-2.817,74	3.000,00	169,06	444,08	1.458,06
AnIC-1000	4.1.	Load the column AnIC-1000. Use 1000 kg of the material in TW-10000. The transfer rate is 2 Bed Volume/h. The resin retains 30% of WATER.	1.000,00	1.000,00	1.006,22	1.006,22	30,19	30,19	1.288,99
	4.2.	Load the column AnIC-1000. Use 2800 kg of the material in Rcat-3000. The resin retains 100% of Copper, 100% of cysteine, 100% of Insulin and 100% of WHEY. Displaced Liquid Stream: The displaced liquid, named mAbani, is sent to Rani-4000.	254,02	1.254,02	255,71	1.261,93	169,06	199,25	1.458,06
	4.3.	Elute the column AnIC-1000 with 2000 kg of the material in TW-10000. Collect 1000 kg at 0 kg into the elution. The cut, named mAbani(2), is sent to Rani-4000. The solvent removes 100% of mAb, 10% of WHEY, 10% of cysteine and 10% of Insulin.	-309,90	944,12	-311,93	1.000,00	120,75	320,00	1.578,80
Rani-4000	4.2.	Load the column AnIC-1000. Use 2800 kg of the material in Rcat-3000. The resin retains 100% of Copper, 100% of cysteine, 100% of Insulin and 100% of WHEY. Displaced Liquid Stream: The displaced liquid, named mAbani, is sent to Rani-4000.	2.545,98	2.545,98	2.562,03	4.000,00	169,06	169,06	1.458,06
	4.3.	Elute the column AnIC-1000 with 2000 kg of the material in TW-10000. Collect 1000 kg at 0 kg into the elution. The cut, named mAbani(2), is sent to Rani-4000. The solvent removes 100% of mAb, 10% of WHEY, 10% of cysteine and 10% of Insulin.	1.001,00	3.546,98	1.007,34	4.000,00	60,44	229,50	1.518,50
	5.1.	Ultrafilter the contents of Rani-4000 in UF-1000. The mode of operation is Feed and Bleed. Recycle 100% of the retentate. The bleed is sent to T-100000. Separation is: 100% of mAb in Liquid goes to the Retentate and 20% of WATER in Liquid goes to the Retentate. 100% of each unspecified component goes to Permeate.	-2.793,72	753,25	-2.811,42	4.000,00	168,69	458,50	1.747,49
	5.2.	Transfer contents of unit Rani-4000 to TmAb-2500. The transfer time is 15 min.	-753,25	0,00	-757,94	4.000,00	15,00	473,50	1.762,49
UF-1000	5.1.	Ultrafilter the contents of Rani-4000 in UF-1000. The mode of operation is Feed and Bleed. Recycle 100% of the retentate. The bleed is sent to T-100000. Separation is: 100% of mAb in Liquid goes to the Retentate and 20% of WATER in Liquid goes to the Retentate. 100% of each unspecified component goes to Permeate.	0,00	0,00	0,00	1.000,00	168,69	168,69	1.747,49
TmAb-2500	5.2.	Transfer contents of unit Rani-4000 to TmAb-2500. The transfer time is 15 min.	753,25	753,25	757,94	2.500,00	15,00	15,00	1.762,49

Step Recipe Description

Process (Version): Purification (1.0)
Step (Version): Purification (1.0)
Simulation Date: 22/5/21 9:19

Key Input Intermediate: mAb
Key Output Intermediate: mAb
Number of Batches: 1
Plan Quantity: 55,38 kg

Sequence Number	Operation	Charged Material	Average Density (kg/Cubic m)	Average Molecular Weight	Unit ID	Vessel Capacity (liter)	Charge Volume (liter)	Charge Mass (kg)	Vessel Liquid+Solid Volume (liter)	Vessel Volume Occupancy (%)	Operation Time (min)	Batch Time (min)	Volume Equivalent wrt Key Input	Weight Equivalent wrt Key Input	Molar Equivalent wrt Key Input
1.1.	Charge T-12500 with 10640 kg of mAbproduction. The charge	Insulin (mAbproduction)	993,81	18,12	T-12500	12.500,00	0,00	0,00	12.500,00	100,00	15,00	15,00	0,00	0,00	0,58
	time is 15 min.	WHEY (mAbproduction)					116,51	115,79					2,00	2,00	16.648,17
		CHO (mAbproduction)					0,01	0,01					0,00	0,00	1,45
		WATER (mAbproduction)					10.531,29	10.466,14					180,78	180,78	1.504.788,41
		mAb (mAbproduction)					58,26	57,90					1,00	1,00	1,00
		cysteine (mAbproduction)					0,15	0,15					0,00	0,00	21,70
		Copper (mAbproduction)					0,00	0,00					0,00	0,00	0,43
1.2.	Filter the contents of T-12500 in depth filter F-10. The transfer rate is 2,25 Cubic m/h. The filtrate, named mAbfiltrate, is sent to R-12500.				T-12500	12.500,00			12.500,00	100,00	285,50	300,50			
					F-10	10,00			10,00	100,00	285,50	300,50			
					R-12500	12.500,00			12.500,00	100,00	285,50	300,50			
2.1.	Charge TW-10000 with 9000 kg of WATER.	WATER	993,81	18,02	TW-10000	10.000,00	9.056,02	9.000,00	10.000,00	100,00	15,00	315,50	155,45	155,45	1.293.990,93
2.2.	Charge TlowpH-10000 with 8000 kg of Low pH tampon.	HYDROGEN-CHLORIDE (Low pH tampon)	993,57	18,03	TlowpH-10000	10.000,00	10,02	7,99	10.000,00	100,00	15,00	330,50	0,17	0,14	567,90
		WATER (Low pH tampon)					8.041,75	7.992,01					138,04	138,04	1.149.065,09
2.3.	Load the column AffC-1000. Use 1000 kg of the material in TW-10000. The transfer rate is 2 Bed Volume/h. The resin retains 30% of WATER.				AffC-1000	1.000,00			1.006,22	100,62	30,19	360,69			
					TW-10000	10.000,00			10.000,00	100,00	30,19	360,69			
2.4.	Load the column AffC-1000. Use 10639 kg of the material in R-12500. The transfer rate is 2 Bed Volume/h. The resin retains 98% of mAb, 5% of cysteine, 5% of Insulin, 5% of Copper, 5% of WHEY and 0,01% of WATER. Displaced Liquid Stream: The displaced liquid, named Residue, is sent to T-100000.				AffC-1000	1.000,00			1.315,84	131,58	321,16	681,84			
					T-100000	100.000,00			100.000,00	100,00	321,16	681,84			
					R-12500	12.500,00			12.500,00	100,00	321,16	681,84			
2.5.	Elute the column AffC-1000 with 2500 kg of the material in TlowpH-10000. The elution rate is 0,5 Bed Volume/h. Collect 2000 kg at 200 kg into the elution. The cut, named mAbAff, is sent to Raff-3000. The solvent removes 98% of mAb, 60% of cysteine, 60% of Insulin, 95% of Copper and 40% of WHEY. Unspecified components remain in the column.				Raff-3000	3.000,00			3.000,00	100,00	248,52	954,52			
					TlowpH-10000	10.000,00			10.000,00	100,00	301,94	983,78			
					AffC-1000	1.000,00			1.257,56	125,76	301,94	983,78			
3.1.	Load the column CatC-1000. Use 1000 kg of the material in TW-10000. The transfer rate is 2 Bed Volume/h. The resin retains 30% of WATER.				TW-10000	10.000,00			10.000,00	100,00	30,19	1.013,97			
					CatC-1000	1.000,00			1.006,22	100,62	30,19	1.013,97			
3.2.	Load the column CatC-1000. Use 2055 kg of the material in Raff-3000. The transfer rate is 1 Bed Volume/h. The resin retains 5% of Copper, 95% of cysteine, 30% of Insulin and 1% of mAb. Displaced Liquid Stream: The displaced liquid, named mAbcat, is sent to Rcat-3000.				Rcat-3000	3.000,00			3.000,00	100,00	124,09	1.138,06			
					Raff-3000	3.000,00			3.000,00	100,00	124,09	1.138,06			
					CatC-1000	1.000,00			1.252,44	125,24	124,09	1.138,06			
3.3.	Elute the column CatC-1000 with 2000 kg of the material in TW-10000. Collect 1000 kg at 0 kg into the elution. The cut, named mAbcat(2), is sent to Rcat-3000. The solvent removes 100% of mAb, 10% of WHEY, 10% of cysteine and 10% of Insulin.				Rcat-3000	3.000,00			3.000,00	100,00	60,42	1.198,47			
					CatC-1000	1.000,00			1.000,00	100,00	120,75	1.258,80			
					TW-10000	10.000,00			10.000,00	100,00	120,75	1.258,80			
4.1.	Load the column AniC-1000. Use 1000 kg of the material in TW-10000. The transfer rate is 2 Bed Volume/h. The resin retains 30% of WATER.				TW-10000	10.000,00			10.000,00	100,00	30,19	1.288,99			
					AniC-1000	1.000,00			1.006,22	100,62	30,19	1.288,99			
4.2.	Load the column AniC-1000. Use 2800 kg of the material in Rcat-3000. The resin retains 100% of Copper, 100% of cysteine, 100% of Insulin and 100% of WHEY. Displaced Liquid Stream: The displaced liquid, named mAbani, is sent to Rani-4000.				AniC-1000	1.000,00			1.261,93	126,19	169,06	1.458,06			
					Rcat-3000	3.000,00			3.000,00	100,00	169,06	1.458,06			
					Rani-4000	4.000,00			4.000,00	100,00	169,06	1.458,06			
4.3.	Elute the column AniC-1000 with 2000 kg of the material in TW-10000. Collect 1000 kg at 0 kg into the elution. The cut, named mAbani(2), is sent to Rani-4000. The solvent removes 100% of mAb, 10% of WHEY, 10% of cysteine and 10% of Insulin.				Rani-4000	4.000,00			4.000,00	100,00	60,44	1.518,50			
					TW-10000	10.000,00			10.000,00	100,00	120,75	1.578,80			
					AniC-1000	1.000,00			1.000,00	100,00	120,75	1.578,80			
5.1.	Ultrafilter the contents of Rani-4000 in UF-1000. The mode of operation is Feed and Bleed. Recycle 100% of the retentate. The bleed is sent to T-100000. Separation is: 100% of mAb in Liquid goes to the Retentate and 20% of WATER in Liquid goes to the Retentate. 100% of each unspecified component goes to Permeate.				T-100000	100.000,00			100.000,00	100,00	168,69	1.747,49			
					Rani-4000	4.000,00			4.000,00	100,00	168,69	1.747,49			
					UF-1000	1.000,00			1.000,00	100,00	168,69	1.747,49			
5.2.	Transfer contents of unit Rani-4000 to TmAb-2500. The transfer time is 15 min.				Rani-4000	4.000,00			4.000,00	100,00	15,00	1.762,49			
					TmAb-2500	2.500,00			2.500,00	100,00	15,00	1.762,49			

Step Recipe Sequence Diagram

Process (version): Purification (1.0)
Step (version): Purification (1.0)
Simulation Date: ##########

Key Input Intermediate: mAb
Key Output Intermediate: mAb
Number of Batches: 1
Plan Quantity: GL_1B

T-12500

Start Time	End Time	Operation Time	Sequence Number		Vessel Liquid Mass	Vessel Liquid Volume	Vessel Temp	Vessel Pressure
[min]	[min]	[min]			[kg]	[liter]	[C]	[kPa]
0,00	15,00	15,00	1.1.	Charge T-12500 with 10640 kg of mAbproduction. The charge time is 15 min.	10.640,00	12.500,00	25,00	101,33
15,00	300,50	285,50	1.2.	Filter the contents of T-12500 in depth filter F-10. The transfer rate is 2,25 Cubic m/h. The filtrate, named mAbfiltrate, is sent to R-12500.	0,00	12.500,00	25,00	101,33

Input Streams	From	Mass [kg]	Volume [liter]	Temp [C]
mAbproduction		10.640,00	10.706,23	25,00

Mass [kg]	Volume [liter]	Temp [C]	To	Output Streams
10.640,00	10.706,23	25,00	F-10	1.2. Depth-Filter-3

F-10

Start Time	End Time	Operation Time	Sequence Number		Vessel Liquid Mass	Vessel Liquid Volume	Vessel Temp	Vessel Pressure
[min]	[min]	[min]			[kg]	[liter]	[C]	[kPa]
15,00	300,50	285,50	1.2.	Filter the contents of T-12500 in depth filter F-10. The transfer rate is 2,25 Cubic m/h. The filtrate, named mAbfiltrate, is sent to R-12500.	0,01	10,00	25,00	101,33

Input Streams	From	Mass [kg]	Volume [liter]	Temp [C]
1.2. Depth-Filter-3	T-12500	10.640,00	10.706,23	25,00

Mass [kg]	Volume [liter]	Temp [C]	To	Output Streams
10.639,99	10.706,22	25,00	R-12500	mAbfiltrate

R-12500

Start Time	End Time	Operation Time	Sequence Number		Vessel Liquid Mass	Vessel Liquid Volume	Vessel Temp	Vessel Pressure
[min]	[min]	[min]			[kg]	[liter]	[C]	[kPa]
15,00	300,50	285,50	1.2.	Filter the contents of T-12500 in depth filter F-10. The transfer rate is 2,25 Cubic m/h. The filtrate, named mAbfiltrate, is sent to R-12500.	10.639,99	12.500,00	25,00	101,33
360,69	681,84	321,16	2.4.	Load the column ARC-1000. Use 10639 kg of the material in R-12500. The transfer rate is 2 Bed Volume/h. The resin retains 98% of mAb, 5% of cysteine, 5% of insulin, 5% of Copper, 5% of WHEY and 0,01% of WATER. Displaced Liquid Stream: The displaced liquid, named Residue, is sent to T-100000.	0,98	12.500,00	25,00	101,33

Input Streams	From	Mass [kg]	Volume [liter]	Temp [C]
mAbfiltrate	F-10	10.639,99	10.706,22	25,00

Mass [kg]	Volume [liter]	Temp [C]	To	Output Streams
10.639,00	10.705,22	25,00	ARC-1000	2.4. Load-Column-15

TW-10000

Start Time	End Time	Operation Time	Sequence Number		Vessel Liquid Mass	Vessel Liquid Volume	Vessel Temp	Vessel Pressure
[min]	[min]	[min]			[kg]	[liter]	[C]	[kPa]
300,50	315,50	15,00	2.1.	Charge TW-10000 with 9000 kg of WATER.	9.000,00	10.000,00	25,00	101,33
330,50	360,69	30,19	2.3.	Load the column ARC-1000. Use 1000 kg of the material in TW-10000. The transfer rate is 2 Bed Volume/h. The resin retains 30% of WATER.	8.000,00	10.000,00	25,00	101,33
993,78	1.013,97	30,19	3.1.	Load the column CarC-1000. Use 1000 kg of the material in TW-10000. The transfer rate is 2 Bed Volume/h. The resin retains 30% of WATER.	7.000,00	10.000,00	25,00	101,33
1.138,06	1.258,80	120,75	3.3.	Elute the column CarC-1000 with 2000 kg of the material in TW-10000. Collect 1000 kg at 0 kg into the elution. The cut, named mAbcarC(), is sent to Ron-8000. The solvent removes 100% of mAb, 10% of WHEY, 10% of cysteine and 10% of insulin.	5.000,00	10.000,00	25,00	101,33
1.258,80	1.288,99	30,19	4.1.	Load the column AnIC-6000. Use 1000 kg of the material in TW-10000. The transfer rate is 2 Bed Volume/h. The resin retains 30% of WATER.	4.000,00	10.000,00	25,00	101,33
1.458,06	1.578,80	120,75	4.3.	Elute the column AnIC-6000 with 2000 kg of the material in TW-10000. Collect 1000 kg at 0 kg into the elution. The cut, named mAbanI(), is sent to Ron-8000. The solvent removes 100% of mAb, 10% of WHEY, 10% of cysteine and 10% of insulin.	2.000,00	10.000,00	25,00	101,33

Input Streams	From	Mass [kg]	Volume [liter]	Temp [C]
WATER		9.000,00	9.054,03	25,00

Mass [kg]	Volume [liter]	Temp [C]	To	Output Streams
1.000,00	1.006,22	25,00	ARC-1000	2.3. Load-Column-12
4.000,00	4.006,22	25,00	CarC-1000	3.1. Load-Column-25
2.000,00	2.012,45	25,00	CarC-1000	3.3. Elute-Column-32
2.000,00	2.012,45	25,00	AnIC-1000	4.1. Load-Column-38
2.000,00	2.012,45	25,00	AnIC-1000	4.3. Elute-Column-45

TlowpH-10000

Start Time	End Time	Operation Time	Sequence Number		Vessel Liquid Mass	Vessel Liquid Volume	Vessel Temp	Vessel Pressure
[min]	[min]	[min]			[kg]	[liter]	[C]	[kPa]
315,50	330,50	15,00	2.2.	Charge TlowpH-10000 with 8000 kg of low pH tampon.	8.000,00	10.000,00	25,00	101,33
681,84	993,78	305,94	2.5.	Elute the column ARC-1000 with 2500 kg of the material in TlowpH-10000. The elution rate is 0,5 Bed Volume/h. Collect 2000 kg at 200 kg into the elution. The cut, named mAbAff, is sent to Raff-9300. The solvent removes 98% of mAb, 60% of cysteine, 60% of insulin, 95% of Copper and 40% of WHEY. Unspecified components remain in the column.	5.500,00	10.000,00	25,00	101,33

Input Streams	From	Mass [kg]	Volume [liter]	Temp [C]
low pH tampon		8.000,00	8.014,78	25,00

Mass [kg]	Volume [liter]	Temp [C]	To	Output Streams
2.500,00	2.514,18	25,00	ARC-1000	2.5. Elute-Column-19

ARC-1000

Start Time	End Time	Operation Time	Sequence Number		Vessel Liquid Mass	Vessel Liquid Volume	Vessel Temp	Vessel Pressure
[min]	[min]	[min]			[kg]	[liter]	[C]	[kPa]
330,50	360,69	30,19	2.3.	Load the column ARC-1000. Use 1000 kg of the material in TW-10000. The transfer rate is 2 Bed Volume/h. The resin retains 30% of WATER.	1.000,00	1.006,22	25,00	101,33
360,69	681,84	321,16	2.4.	Load the column ARC-1000. Use 10639 kg of the material in R-12500. The transfer rate is 2 Bed Volume/h. The resin retains 98% of mAb, 5% of cysteine, 5% of insulin, 5% of Copper, 5% of WHEY and 0,01% of WATER. Displaced Liquid Stream: The displaced liquid, named Residue, is sent to T-100000.	1.307,70	1.315,84	25,00	101,33
681,84	993,78	305,94	2.5.	Elute the column ARC-1000 with 2500 kg of the material in TlowpH-10000. The elution rate is 0,5 Bed Volume/h. Collect 2000 kg at 200 kg into the elution. The cut, named mAbAff, is sent to Raff-9300. The solvent removes 98% of mAb, 60% of cysteine, 60% of insulin, 95% of Copper and 40% of WHEY. Unspecified components remain in the column.	1.249,55	1.257,56	25,00	101,33

Input Streams	From	Mass [kg]	Volume [liter]	Temp [C]
2.3. Load-Column-12	TW-10000	1.000,00	1.006,22	25,00
2.4. Load-Column-15	R-12500	10.639,00	10.705,22	25,00
2.5. Elute-Column-19	TlowpH-10000	2.500,00	2.514,18	25,00

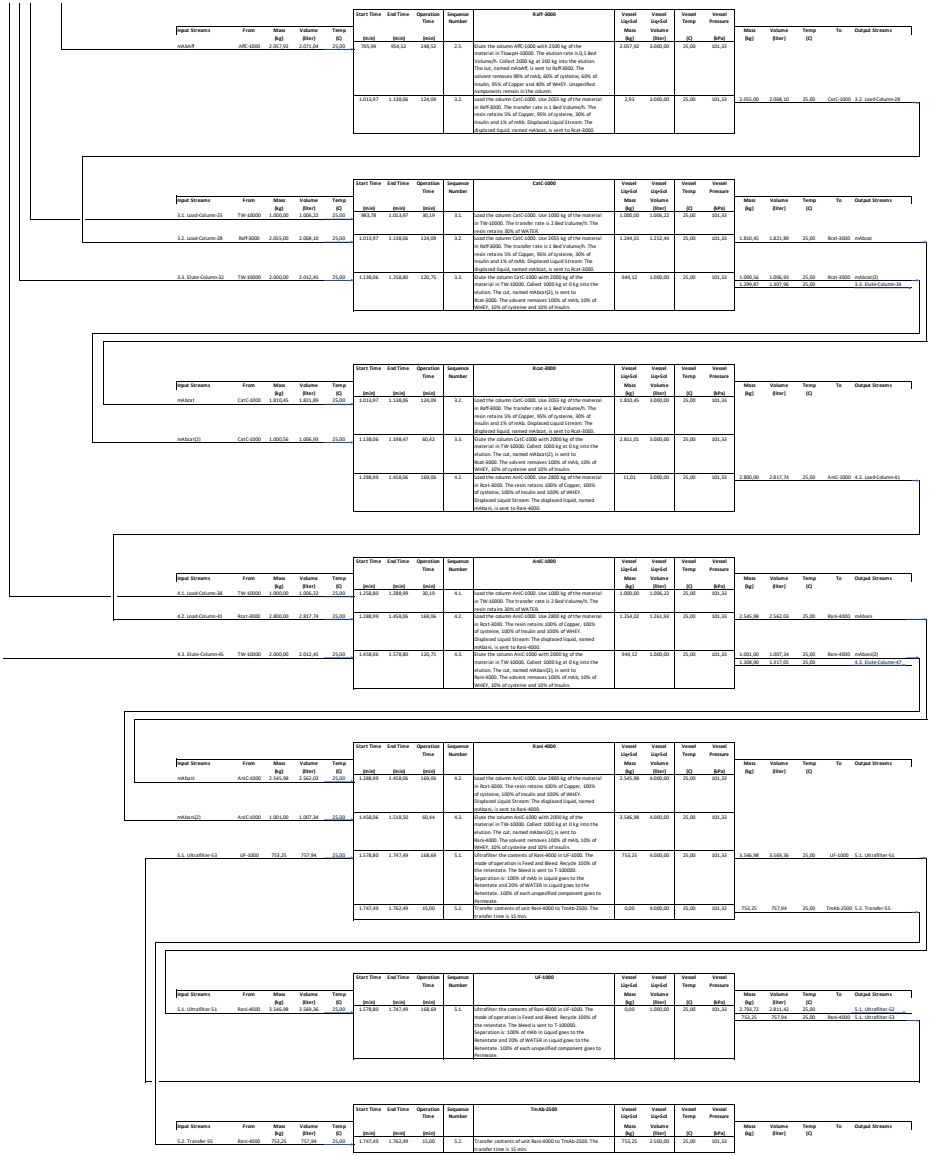
Mass [kg]	Volume [liter]	Temp [C]	To	Output Streams
10.191,30	10.395,61	25,00	T-100000	Residue
200,00	201,24	25,00		2.5. Elute-Column-20
2.017,92	2.073,18	25,00	Raff-9300	mAbAff
300,23	302,18	25,00		2.5. Elute-Column-22

T-100000

Start Time	End Time	Operation Time	Sequence Number		Vessel Liquid Mass	Vessel Liquid Volume	Vessel Temp	Vessel Pressure
[min]	[min]	[min]			[kg]	[liter]	[C]	[kPa]
360,69	681,84	321,16	2.4.	Load the column ARC-1000. Use 10639 kg of the material in R-12500. The transfer rate is 2 Bed Volume/h. The resin retains 98% of mAb, 5% of cysteine, 5% of insulin, 5% of Copper, 5% of WHEY and 0,01% of WATER. Displaced Liquid Stream: The displaced liquid, named Residue, is sent to T-100000.	10.331,30	100.000,00	25,00	101,33
1.578,80	1.747,49	168,69	5.1.	Ultrafilter the contents of Ken-4000 in UF-5000. The mode of operation is Feed and Bleed. Recycle 100% of the retentate. The bleed is sent to T-100000. Separation is: 100% of mAb in liquid goes to the Retentate and 30% of WATER in liquid goes to the Retentate. 100% of each unspecified component goes to Permeate.	10.331,30	100.000,00	25,00	101,33

Input Streams	From	Mass [kg]	Volume [liter]	Temp [C]
Residue	ARC-1000	10.331,30	10.395,61	25,00

Mass [kg]	Volume [liter]	Temp [C]	To	Output Streams


Raff-3000
CarC-1000
Rcat-3000
AniC-1000
Rani-4000
UF-1000
TmAb-2500