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Single-Use Systems Bioreactors in the Biopharmaceutical Industry and its Use in SARS-CoV-2 Candidate Vaccine Production - A Review

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Abstract

Single-Use-Systems (SUSs) Bioreactors, also known as disposable bioreactors, have become an integral part of biotechnology manufacturing facilities for bioproducts with a potential market expecting a growth rate of nearly 15.5% over the forecast period: 2018 to 2023. SUSs bioreactors are comparatively safe, simple, and flexible than their stainless-steel bioreactors counterparts thus, their usage is being augmented in the biopharmaceutical industry mainly in planning fast tracks of complex projects, including those related to the SARS-CoV-2 pandemic. Thus, the use of SUSs has become an effective alternative for the rapid production of vaccine candidates. However, some technical and operational disadvantages still hamper their worldwide use. This review gives a rational insight into SUSs bioreactors use, types, operational parameters and new applications in the biopharmaceutical industry. Likewise, the appropriate parameters and limitations of this equipment, focusing on its use for vaccine production against COVID-19 are also discussed.

Keywords: Single-Use Bioreactor Systems; SARS-CoV-2; Viral Vaccine Production; Scale-Up; Biopharmaceutics

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Introduction

According to the growing global demand, it is clear that next-generation vaccines will be produced using recombinant organisms and Single-Use Systems (SUSs) including SUSs bioreactors addressed to increase production capacities with reduced costs. SUSs devices, in general, refer to bioprocessing equipment designed to be used once or in a single production campaign and then discarded [1].

The description above can be evidenced in the SARS CoV-2 pandemic. The worldwide presence of SARS-CoV-2 affected almost all industries and social functions globally, including the biopharmaceutical industry, and with it the bioprocesses for vaccines or its candidate's production [2,3]. This fact has brought, promptly, a series of significant changes that include existing production practices and/or some preexisting trends aimed at an adaptation process to face this dramatic new situation.

Therefore, the new technology in this pandemic contributes to the rapid development and production of candidate vaccines, therapies, equipment, and diagnostics, all at the same time. Also, according to Kulkarni S (2019) [3], and Macdonald GJ (2020) [4], the changes that this global resource mobilization will bring to the biopharmaceutical industry include:

1. Single-use systems, there will be more SUSs in the new process lines and facilities;
2. Process intensification, faster bioprocessing with smaller facilities and less staff;
3. Greater automation, Plug and Play technology which allows switching between batches, with single-use bioprocessing, five times faster than the traditional process with a stainless steel environment; and
4. Greater biosecurity, security for both, production operations and research personnel.

Assuming only two or three pandemic-related vaccines or biotherapeutics are entering the market, each one will likely require the manufacture of hundreds of millions, if not billions, of doses per year. This will require multiple installations. For example, only vaccine manufacturing could involve 4,000 SUSs/year with a total production of eight million liters of culture media per year [4].

It is considered that SUSs demand, including SUSs bioreactors, will increase with the pandemic. It is also true that, since some years ago there was a significant increase of SUSs use in the biopharmaceutical industry, especially in the production of proteins from mammalian cell cultures [5].

It has also been proven that with the use of SUSs, for example in mammalian cell cultures titers, production can be increased from mg / L to g / L by improving the bio-manufacturing of SUSs, which has reduced goods cost during the last 30 years [6].

SUSs bioreactors versatility and potential capabilities positions them as an ideal system in the bioprocess industry as an alternative to conventional glass and stainless steel bioreactors, offering greater flexibility in the bioprocess and ease of operations [7-9].

This work provides, for the first time, an updated overview to academics and researchers, pointed to the use of single-use devices, specifically disposable bioreactors, in the production of viral vaccines, other biopharmaceutical applications and possible challenges of SUSs bioreactors in the race for the production of vaccine candidates in the fight against COVID -19 worldwide.

What are single-use systems (SUSs) devices?

SUSs include upstream or downstream devices such as bioreactors, membranes, mixing systems, connectors, buffer containers, etc. They are mainly composed of plastic material or other biodegradable biopolymers sealed and sterilized by radiation [10]. Figure 1 shows the trend in the use of different disposable components for bioprocessing between 2018 and 2019, including SUSs bioreactors.

Market behavior, as shown in Figure 2, shows a preference for SUSs use. SUSs bioreactors preference in bioprocesses has been increasing since 2013 with a growing projection to 2023 when compared with its stainless-steel counterparts (Figure 3).

Single-Use Bioreactors vs. Conventional Bioreactors

Among the indisputable operational and productive advantages of SUSs bioreactors we can find:

Production time saving, since SUSs eliminate the need for re-sterilization and validation of cleaning systems, which ensures a lower cross-contamination risk [11].

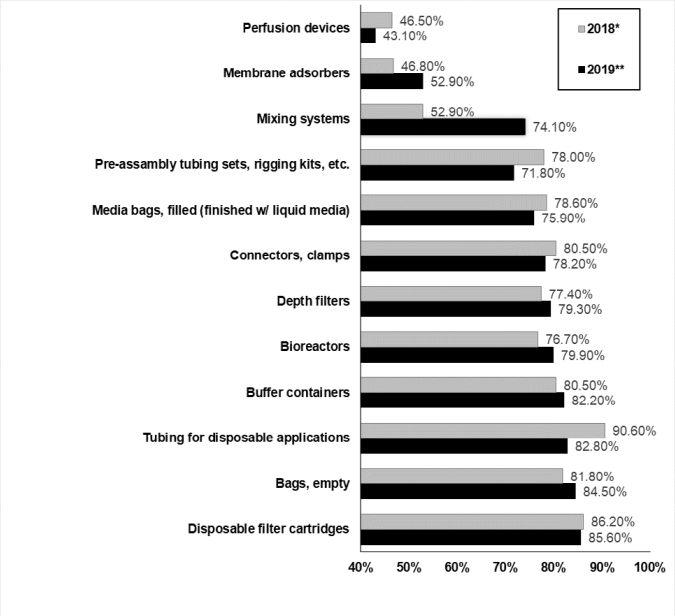

Figure 1: Trend towards the use of disposable single-use elements in bioprocesses 2018-2019.

Sources: 15th* and 16th** Annual Report and Survey on Biopharmaceutical Manufacturing April 2018 and 2019 respectively.

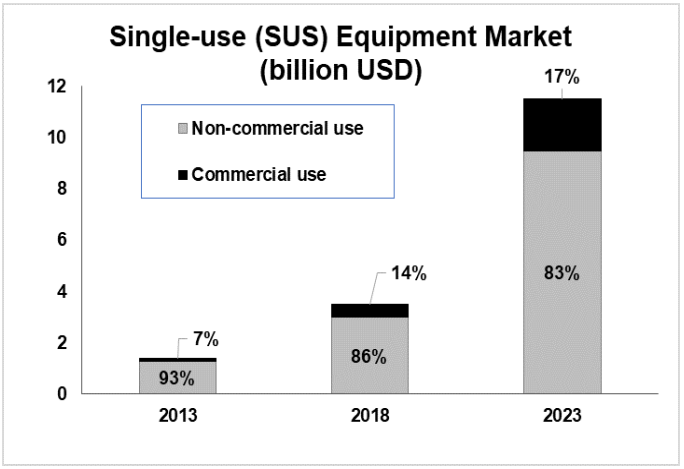

Figure 2: Projection of single-use systems bioreactors market 2013-2023.

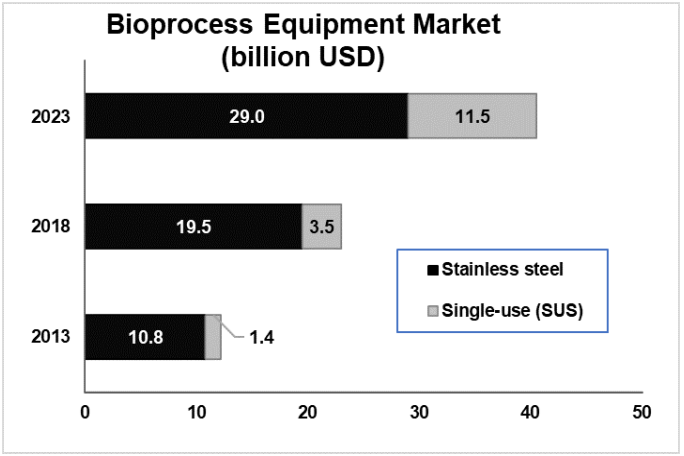

Figure 3: Market comparison of stainless steel vs SUSs 2013-2023.

Low operational costs and reduced fixed capital investment because of the limited manpower and infrastructure needed [11].

The final product can enter the commercialization process more expeditiously compared to the products obtained using conventional bioreactors [11,12].

They allow fast and flexible manufacture. For that reason, SUSs are used to accelerate commercialization in the production of antibodies, vaccines, and therapeutic cells [13]. Consequently, SUSs have an advantage over stainless steel bioreactors for large-scale production of vaccines. Thereby, figure 4 shows some limitations of conventional bioreactors such as cross contamination, high water waste, a need for sterilization process, large production times and high cost and energy. These characteristics create a tendency for the biopharmaceutical industry to migrate towards a more flexible and agile production using SUSs.

Main Types of SUSs Used in Biopharmaceutical Applications

Bioreactor design has evolved to meet technical requirements for vaccine manufacturing since the early 1940s when the use of microorganisms in vaccine manufacturing was the main practice, until the use of mammalian cells. Mammalian cells had become a technical

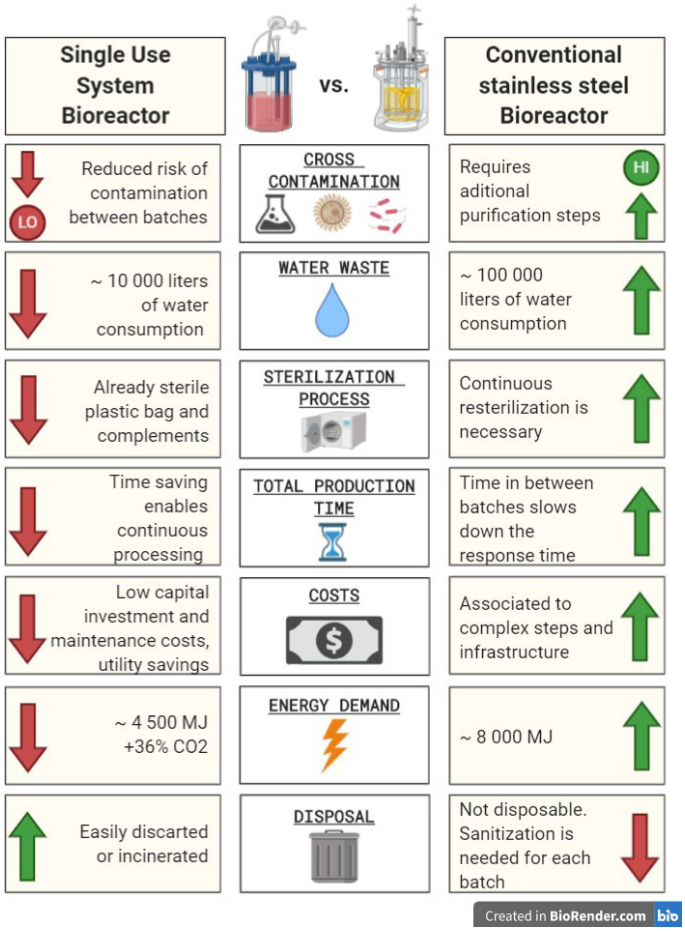

Figure 4: Advantages of Single-Use Systems over conventional bioreactors using different operational and productive parameters.

challenge for several companies, forcing them to continuously develop innovative products and workflows [14]. As a result, SUSs designs respond to the changing needs not only for the manufacture of vaccines but also for the production of cells, vectors, recombinant proteins, antibodies and secondary metabolites. In this regard, different systems are being designed to achieve these objectives, some of the most used in the biopharmaceutical industry are described below.

Stirred Tank SUSs Bioreactors

Single-use stirred tank bioreactors are a viable alternative for culturing animal cells because of their high throughput, flexibility, and low cross-contamination. They consist of a plastic container with a built-in impeller, contained within a steel tank that facilitates heat transfer [15].

Agitated tank SUSs have successfully replaced their stainless-steel counterparts. Kazemzadeh A, et al. (2018) [16], evaluated the effect of gas volumetric flow rate, impeller speed on shear rate, gas retention and bubble diameter for an angular-axis SUSs stirred tank bioreactor. This study also determined that the mixing system is similar to that of conventional stirred tank bioreactors and meets the requirements for culturing cells.

Currently, the two main manufacturers of this type of SUSs are Thermo-Fisher and Xcellerex and the main commercial brands are AllegroTM STR200, BioBLU®, bioReactor, BIOSTAT® Cultibag STR200, CerCell, iCELLis® fixed-bed bioreactor, Mobius® CellReady, Sartorius Ambr®, SmartVessel™ / HyPerforma™, and Xcellerex™ XDR [16].

Vaccine development, viral vectors and cell growth are the most recent application of this type of SUSs. Also, it has been proven that the combination with microcarriers in the system provides good results [17]. Lawson T, et al. (2017) [18], achieved a 43-fold expansion of human mesenchymal stromal cells (hMSC) by incorporating microcarriers in the bioreactor. The expanded cells showed inhibition of T cell growth and inducibility of indoleamine 2,3- dioxygenase. A similar study was conducted by Dufey V, et al. (2016) [19], who used the

BioBLU® 0.3c system and generated 1×10^8 cells in a 250 mL working volume. On the other hand, Yang J, et al. (2019) [17], developed an expansion process with microcarriers for HEK293T and Vero cells growth, obtaining a maximum cell concentration of 1.5×10^6 cells / mL and 3.1×10^6 cells / mL with the XDR-50 and XDR-200 bioreactors respectively.

Other successful studies have shown the potential of these SUSs for the production of viral vaccines, and vectors. In 2019, Fedosyuk S, et al. (2019) [20], manufactured vaccine candidates against rabies, malaria and Rift Valley fever, using a type of adenovirus in a single-use stirred tank bioreactor.

Oscillating SUSs Bioreactors

Oscillating wave bioreactors are easy to use and mainly suitable for shear sensitive cell cultures, also displaying comprehensive options for process control and monitoring. Therefore, it has several useful applications for example in cell bank processes and seed inocula within the upstream bioprocess for various cell types: CHO cells, NS0 cells, Escherichia coli, stem cells in clinical applications, insect cells, Madin-Darby Canine Kidney cells (MDCK), HEK 293, algae and cyanobacteria [11,21, and 22].

These type of SUSs generally consist of a biocompatible polymer bag placed on a rocker unit which enables the medium of the bag to be gently mixed through rocking motion [23]. Some of the available platforms include: Biostat® RM, Flexsafe® RM Bags (Sartorius AG) and Wave Bioreactor™ (Cytiva, GE, US) [24].

However, there are certain limitations in the characterization of oscillating bioreactors, mainly from the lack of information. The mass transfer of gas-liquid oxygen in these systems depends on the surface aeration with the air drag of the breaking waves during the forward and backward rolling action [11]. The energy input on gas-liquid mass transfer and mixing in the bioreactor is unknown. Bai Y, et al. (2019) [11], determined that the gas-liquid mass transfer and mixing time decrease with increasing energy input. Still, a more detailed investigation is necessary to gain more technical information.

Due to its rapid process development and clinical manufacturing characteristics, the oscillating bioreactor can be used in cell-based immunotherapy. Meng Y, et al. (2018) [21], took advantage of this kind of bioreactor in the production of peripheral blood mononuclear cell (PBMC), Cytokine-induced killer cells (CIK), Natural Killers (NK), and Dendritic cells (DCs) for clinical trials [21]. The achieved results were promising for cell expansion, generating effective antineoplastic CIK and NK cells; which is an alternative for the production of mammalian cells on a large scale under good manufacturing practices conditions with useful therapeutic applications [21].

The baculovirus expression system is widely used for the production

of recombinant proteins because of its versatility and high level of protein expression. Given the advantages of using this expression system, Ghasemi A, et al. (2019) [23], analyzed the implementation of a single-use wave bioreactor for the expression of human papillomavirus L1 protein in infected/uninfected insect cells, obtained from Spodoptera frugiperda (Sf9) and derived from Trichoplusia ni (Tn-5). The effect of the specific oxygen absorption rate (SOUR) was evaluated to scale the process. The results obtained the highest SOUR values after Tn-5 cell infection. The wave bioreactor system (GE, US) used did not use bubbles, which demonstrated minimal shear impact on cells [23].

The application of single-use bioreactors studied in fungal cultures is of great interest due to the ability to synthesize secondary metabolites with biological activity. Aspergillus niger is a filamentous fungus with the ability to synthesize non-ribosomal peptides that show antibacterial, antifungal, insecticidal, anthelmintic, and anticancer activities [25]. Kurt T, et al. (2018) [25], evaluated the heterologous production of the commercial drug enniatin B and the hydrodynamic conditions of A. niger cultivated in a disposable two-dimensional rocking motion bioreactor (CELL-tainer®) compared to a conventional stirred bioreactor (BioFlo STR). It was observed that enniatin B titers obtained with BioFlo STR were higher than those obtained with CELL-tainer®. Although the values were not competitive with the conventional system, the use of these SUSs can be considered for the culture of mutant strains sensitive to shear, as well as for obtaining high cell density cultures or fast biomass acumulation [25].

In the pharmaceutical applications of oscillating SUSs using plant cells, Khojasteh A, et al. (2016) [26] studied the cell suspension cultures of Satureja khuzistanica, which were evaluated for the production of rosmarinic acid (RA), a secondary metabolite with biological activity in slowing down the development of Alzheimer's and as an anti-cancer agent. A mechanically driven single-use bioreactor (BIOSTAT RM 20/50 from Sartorius Stedim Biotech) was used for the study with a maximum RA production of 3.1 g / L and a biomass productivity of 18.7 g / L with methyl jasmonate as an inducer [26].

Also, Halgo D, et al. (2017) [27], evaluated the plant cell culture of Centella asiatica. This plant is used in traditional medicine for the treatment of dry skin conditions, leprosy, varicose ulcers, eczema and / or psoriasis due to its centelosides. The use of a 2L CellBag (GE Heathcare Bio - Sciences AB, Uppsala, Sweden) resulted in a successful growth of C. asiatica cell line, achieving a growth index of 4.8 with a final production of centeloside of 7.3 mg / g dry weight using combined treatments with inducers, crop feeding and natural sources of amyrins.

Perfusion SUSs Bioreactors

The implementation of continuous operations, also called perfusion systems, can obtain expensive biological products in small volumes. These systems are limited because they require continuous feeding of new media and disposal of the used media. In this SUSs model, cells are grown in a plastic bag where cells are retained in the bioreactor through the use of perfusion equipment such as alternate tangential flow devices, cross flow filters, centrifuges, settlers, rotating filters, hydrocyclones or by the use of hollow capillary fibers, flat plates, membranes and microcarriers. The advantages of this system are the ease of continuous culturing of cells due to the lower possibility of accumulation of waste products or filter clogging, flexibility, low cost, improved quality, speed, minimization of the probability of any product inhibition and high product yield [6,28, and 29].

These SUSs are used successfully in the production of complex proteins/enzymes [6]. They provide good nutrient distribution and excellent oxygen transfer without gas bubbles or shear forces, which make them suitable for anchorage-dependent cell line cultures and virus production [6]. There are many manufacturers in the development of these systems such as Pall Life Sciences, Applikon Biotechnology, Satorius, AcuSyt, GE Healthcare, PerfuseCell™, with trademarks like ambr® 250 perfusion, CellMembra™, CellRetention™, CellTernate™ [28,31].

In one of its applications (2020), three processes for the manufacture of monoclonal antibodies produced by Chinese hamster ovary (CHO) cells were compared [6]. The used scheme achieved a substantial improvement in titration through intensified seed culture strategies. Besides, these systems can also achieve a rapid and economical process for the production of adenoviruses under good manufacturing practices conditions [30].

Also, Coronel J, et al. (2019) [32] evaluated influenza A virus production in a single-use orbital shaken bioreactor with perfusion systems. The study demonstrated that the cell line used, AGE1. CR1pIX, in suspension obtained large cell densities (50×10^6 cells / ml), cell viability and short doubling time using tangential flow filtration (TFF) and alternating tangential flow filtration (ATF).

Fixed Bed SUSs Bioreactors

Fixed-bed SUSs are highly automated and allow process scaling [33]. Due to their low shear system, they have been used successfully to cultivate mammalian cells and have potential applications for vaccine manufacture [8,33, and 34]. Trademarks of this type of SUSs include: iCELLis®, developed by PALL; Bioreactor scale X, from Univercells; BelloCell® (laboratory scale) and TideCell002 (industrial scale), both from Cesco Bioengineering. The parts of Fixed Bed SUSs bioreactors are: thermocouple, feed line, preheating zone, electrical element, alumina jacket, grid, quartz beads, catalyst bed, asbestos, fire brick, thermocouple, furnace outer wall, and product line [35].

iCELLis® SUSs, for example, are compact, fixed-bed bioreactors with an integrated perfusion system. High yields of viral vaccines, recombinant proteins, adeno-associated viral vectors and retroviral vectors have been produced in these bioreactors [33,34]. Valkama AJ, et al. (2018) [34], tested clinical scaling with the PALL iCELLis® bioreactor, and 293T adherent cells for the production of lentiviral vectors, optimizing the production parameters.

On the other hand, scale-X bioreactors have a variety of growth surfaces: scale-X 'hydro' (< 3 m2), 'carbo' (10-30 m2) and 'nitro' (200-600 m2) [33]. Nogueira DES, et al. (2019) [36], used the scale-X™ carbo system to produce a recombinant vaccine against vesicular stomatitis virus using Vero cells. The results detailed a 4-log increase in virus production when using the bioreactor, demonstrating higher virus production per surface area compared to classical production. In addition, the scale-X carbo bioreactor avoids the limitation of suffering a decrease in production efficiency in scaling up, that other fixed-bed bioreactors have, by increasing the height of the bed design while maintaining the diameter to limit the impact.

Also, BelloCell and TideCell002 SUSs, were used in the cultivation of MDCK and Vero cells for the production of vaccines against H5N1 and H7N9 viruses [20]. The data showed that TideCell002 can produce 10-20 L of vaccine per practice and that the process is 20 times higher in volume compared to the BelloCell-500A® system. Due to the linear scaling of the TideCell002 system, researchers consider it easy for scaling to 500-1000L bioreactors.

Vertical Wheel Sus Bioreactors

Single Use Vertical Wheel Bioreactors (SUVW) are available in a wide range of scales, from 100 mL to 80 L. Agitation in these bioreactors is provided by a large vertical impeller, which when combined with a U-shaped bottom allows better homogenization of the content with a reduced energy input [36].

SUVWs have been successful in the production of various cells such as human mesenchymal stem cells (MSCs), embryonic stem cells (ESC), pluripotent stem cells (PSC), and chondrocytes. As a very significant advantage of SUVWs, compared to traditional bioreactors, cells are exposed to lower shear levels [36,37].

Besides, SUVWs have been successfully used for the expansion of human-induced pluripotent stem cells (hiPSC) as floating aggregates because they show a promising alternative for drug industry and cell-based therapies. To carry out this experience, Nogueira DES, et al. (2019) [36], filled a PBS MINI 0.1 (PBS Biotech) bioreactor with 60 mL of working volume. Two different culture media, mTeSR1 and mTeSR3D, were tested, demonstrating that mTeSR3D supports the expansion of hiPSC stem cells, although with lower cell density than when mTeSR1 was used. In addition, media supplemented with dextran sulfate allowed to obtain a higher cell density with one less day of culture. Therefore, according to the obtained results, SUVWs can be considered as a promising technology for the bioprocessing of hiPSC.

General Parameters to be Considered for Vaccine Production and Other Biopharmaceutical Applications Using SUSs

The steady increase in the manufacture of biologics, the patent expiration for the use of highly successful drugs / molecules, as well as the limited number of potential products in the pipeline, have motivated companies to adopt new techniques and technologies. SUSs are a viable solution for which, different parameters, such as: type of host cells to be used, type of SUSs and operational variables, have been established by different authors to meet the desired results as shown in Table 1 and Table 2:

SUSs update in the production of vaccine candidates against SARS-CoV-2

By the end of 2019, China reported the presence of the severe acute respiratory syndrome SARS-CoV-2 [48]. Therefore, in March 2020, due to its rapid worldwide spread, it was declared a pandemic by the World Health Organization (WHO).

Since then, several vaccine formulations against COVID-19 have been developed and registered mainly in Asia, Europe and North America since August 2020 [49]. Also Latin America joined the race of developing an appropriate vaccine candidate [50,54]. In all such research and production efforts, SUSs devices, including SUSs bioreactors are being applied as a cutting edge technology in the clinical production of biopharmaceuticals [55].

To generate rapid results against the COVID-19 pandemic, the U.S Department of Health and Human services through the Advanced Biomedical Research and Development Authorization (BARDA) created a public-private partnerships called Centers for Innovation in Advanced Development and Manufacturing (CIADM) in order to develop countermeasures to medical emergencies [56].

The Bayview campus of Emergent Biosolutions was selected as one of the three CIADMs due to its experience in the employment of single-use technology for rapid, high-volume manufacturing of vaccines and therapeutics including those related to SARS-CoV-2 [56].

Table 1: General parameters and variables to take into account for Single-Use Systems bioreactors use.

PARAMETER	EQUATION AND VALUES	BIOREACTOR TYPE	APPLICATION	REF.
Reynolds number for the liquid phase, $RRee(LL)$	$RRee(LL)=\omega\omega\ LL2vvLLssssss\ (\alpha\alpha)$ Ec. 1 L is the length of the culture bag, $vvLL$ is the kinematic viscosity of the liquid phase and α is an operational parameter Values between 2.0 × 104 to 1.5 × 105 The need of low shear levels is measured by low Reynolds number values.	Ready To Process Bioreactor WAVETM 25 Aerated stirred bioreactor	Scale-up of bioprocesses focused on non-adherent animal cells	[16,38]
Oxygen transfer coefficient, $KKLLLL$	$KKLLLL$=13,423$RReeLL$0,522$RReeGG$0,664$SSCC$0,572[SS−1] Ec. 2 $RRee(GG)$ is Reynolds number for the gas phase, $SScc$ is Schmidt's number for the liquid phase and $RReeLL$ is the diffusivity coefficient of oxygen in the liquid phase. Values between 2.83-13.55 h-1			[38]
Stirring	Viral vectors: 50 rpm	WAVE	Support expansion of cells and scale-up	[30,38]
Temperature, T°	hMSC cells: 37º C	AmProtein Current Perfusion Bioreactor and Bioflo310® bioreactor		
	Viral vectors: 33º C			
Dissolved oxygen, DO	hMSC cells: 80%			
	Viral vectors: 55%.			
pH	hMSC cells: 7.4 and 7.6			
	Viral vectors: 7.2-7.4			
Volume demand for oxygen, $QQ(OO2)$ and Heat transfer (HER)	$QQ(OO2)=\mu\mu\mu\mu YY(OO2)$ Ec. 3	SUSs	Theoretical and practical considerations for the design and operation of single-use fermenters for microbial cells and their extension, with the aim of using single-use bioreactors in microbial fermentations	[39]
	$HHHHH$ =0,12$QQ(OO2)$ Ec. 4			
	HER: heat evolution rate Stirring 350 rpm Aeration : None HER: 5587 BTU/h (1637 W)			
Growth rate of a crop/ oxygen uptake	Yeast: 130 - 140 OD Pseudomonas: 200 - 370 OD E. coli: 150 - 160 OD and 30% dissolved oxygen			
Energy input	$PPVV=NNppnn3\rho\rho dd3VV$ Ec. 5 P/V is the energy input per volume, P is the power input (W/m3), V is the volume (m3), Np is the Newton number of the impeller or the power number of the impeller, n is the stirring speed (r/s), ρ is the density of the culture medium (kg/m3), d is the diameter of the impeller (m)	WAVE 25 Bioreactors	Determining a suitable stirring rate for HEK293T and Vero cell culture in bioreactors is key to the scaling process	[17]

Table 2: Cell types produced in various applications using Single-use Systems bioreactors.

CELLS TYPES	FEATURES	BIOREACTOR TYPE	APPLICATIONS	REF.
MSCs	Mesenchymal stromal cells: Multipotent and immunomodulatory adult cells. They are derived from adult tissues, bone marrow, adipose tissue, the umbilical cord, and the placenta.	Biorreactor Mobius ® 50 L single-use stirred tank	Cell therapy	[18]
HL-60 cell line	Human acute promyelocytic leukemia cells: Non-adherent hematopoietic cells are sensitive to hydrodynamic effects in cultures.	WAVE 25 Bioreactor with ReadyToProcess WAVE ™ 25 System	Studies on the cytotoxic effects of possible antileukemic drugs. Gene therapy and as a source of blood-derived products.	[38]
CHO	Chinese hamster ovary cell: Cell growth in culture can be affected by leached (toxic) compounds, such as bis (2,4-di-tert-butylphenyl) -phosphate or bDtBPP.	WAVE bioreactors	Production of recombinant therapeutic proteins.	[22,37]
VERO	Continuous African green monkey kidney cell lines: They depend on the anchor for their growth and are susceptible to a wide range of viruses.	BelloCell (tabletop scale) and TideCell (industrial scale)	Production of H5N1 and H7N9 influenza viruses for the vaccine manufacture.	[8,33, and 40]
			Production of a live recombinant vaccine against vesicular stomatitis virus containing the glycoproteins of Lassa fever virus.	
			Production of vaccines against rabies, poliomyelitis and enterovirus.	
HEK293	Human kidney embryonic cells: Used for more than 30 years. They grow in suspension and in a serum-free environment at cell densities up to 20 million cells/ml	XDR-50 ACP perfusion bioreactor B BIOSTAT®STR XcellellerexTMXDR-10 ReadyToProcecess WAVETM25	Production of Covid-19 vaccines: Ad5-nCoV (viral vector vaccine) and ChAdOx1 (recombinant Chimpanzee adenovirus vector vaccine)	[17,30,41, and 43]
AGE1.CR.pIX	culture media.Avian Muscovy duck cell line: Can grow in scalable suspension cultures and adapt to chemically defined	OSB SB 10-X Shaker Orbital Bioreactor coupled with perfusion system	Production of Influenza A virus vaccine	[32,44]
hiPSC	Human-induced pluripotent stem cells are cells derived from the reprogramming of adult somatic cells.	PBS MINI vertical wheel bioreactor	Production of antitumor vaccines in personalized medicine	[36]
Meg01	Human bone marrow megakaryoblasts from chronic myelogenous leukemia: Megakaryocyte (MK) progenitor cell line isolated from the bone marrow of patients with leukemia.	Orbital shaking bioreactor	Production of specific antigens and vaccines against dengue virus infection	[45]
MDCK	Madin-Darby canine kidney cells have well-defined cell connections, a rapid growth rate and are suitable for microscopic studies.	BelloCell y TideCell Oscillating bioreactors	Production of candidate vaccines for H5N1 clade II and H7N9 viruses	[8,46]
ExpiSf9	Insect cell line adapted for high- density suspended growth	Cytiva 22 L Cellbag™ Disposable Bioreactor	Production of recombinant proteins from Baculovirus vectors	[47]

Emergent Biosolutions announced the development and manufacture of its COVID-19 vaccine candidates in collaboration with the Johnson & Johnson Company, which investigated genetically modified adenovirus-based vaccines against SARS-CoV-2 [57]. They also settled an agreement with Novavax for the manufacture of a vaccine candidate and with Vaxart in the development of an oral vaccine for COVID-19 [58]. On June 11, 2020, Emergent BioSolutions declared the provision of its services by using single use technology for the large-scale development and the manufacturing of AstraZeneca's AZD1222 vaccine candidate, a viral vector-based vaccine that contains genetic material from the SARS-CoV-2 Spike (S) protein [57].

On the other hand, CanSinBio Biologics Inc. and the Institute of Bioengineering of the Academy of Military Medical Sciences used the single-use bioreactor BIOSTAT® STR for the production of the recombinant adenovirus vaccine Ad5-nCoV due to its advantages in bioprocess development time, scalability, bioprocess flexibility and cross contamination risks reduction [59]. The Biostat STR® system includes a BioPAT® toolbox that monitors the process and the Flexsafe®STR single-use bags that range from 50 L to 2000 L [60,61]. The process of production for the Ad5-nCoV vaccine is described in figure 5.

The process begins with the optimization of the peak protein gene with UpGene software. This gene is subsequently cloned into a shuttle plasmid of AdMax adenovirus system (Microbix Biosystem, Canada) and co-transfected into HEK293 cells. Finally, for the high production of recombinant particles, cells are amplified using the single-use bioreactor BIOSTAT® STR from Sartorius.

Additionally, the Virology laboratory and the Bioprocess Engineering Group at Wageningen University use SUSs from Applikon Biotechnology for SARS-CoV-2 vaccine production. Their vaccine candidate is based on the production of the viral S protein using baculovirus and Sf9 insect cells as hosts. Their studies consisted of determining the optimal conditions for cell growth and protein production in Sf9 cells. The micro-Matrix system (Applikon Biotechnology, Netherlands) was used for the control of DO, pH and temperature because it offers a total control of 24 independent bioreactors. Optimal conditions were also verified in the miniBio (single-use autoclavable bioreactor) of 500 mL and the AppliFlex ST SUSs bioreactor of 500 mL, which presents scalability characteristics (scalable to 20 L), is customizable, data generates in less time, has simple configuration of its operation and its flexible to the demands of the process [62,63]. However, in some studies, SUSs have been scaled up to 2000 L, but it could be a limitation if a volume greater than 2000 L is required [5,13]. Reactor brands that work with volumes of 2000 L are BIOSTAT ® Cultibag STR200, Mobius ® CellReady and Xcellerex XDR™ [5]. The problems for achieving a scale-up greater than 2000 L focus on the structural soundness of the reactors and the leaks, but this approach is still under discussion.

However, ABEC, a leading global provider of pharmaceutical product development solutions and services, delivered SUSs for the vaccine manufacturing of Novavax's COVID-19 candidate, NVX-CoV2373, which consisted of six 4000 L Custom Single Run (CSR) bioreactors donated to the Serum Institute of India. The aim of the application of these SUSs was to promote a supply of NVX-CoV2373 vaccine throughout India and low/middle income countries doubling its productivity at a lower cost [64].

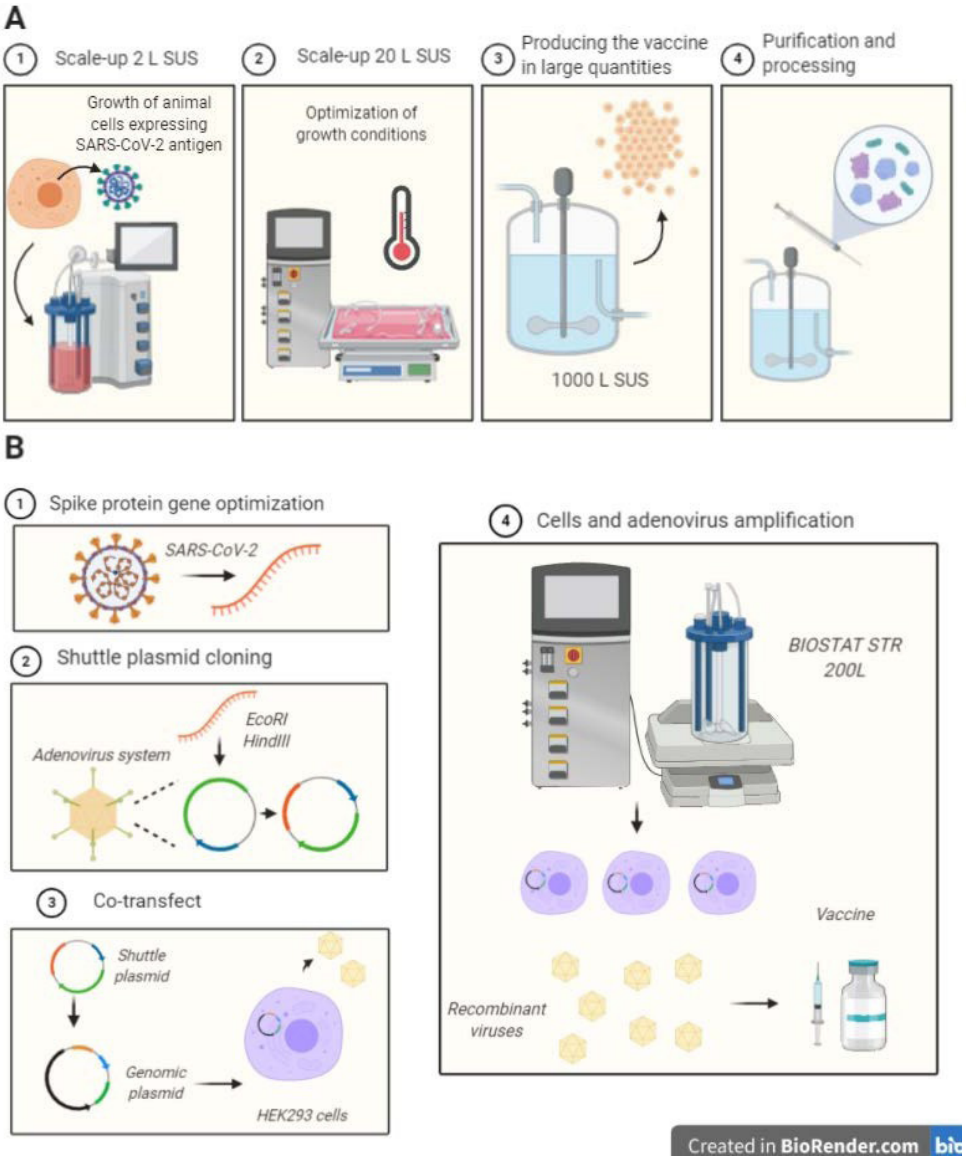

Figure 5: Strategies for COVID-19 Vaccine production by the use of SUSs systems, including SUSs bioreactors. A) General process for vaccine production applied to some candidates against SARS-COV-2. B) Production of the Ad5-nCoV non-replicating viral vector vaccine.

Final Considerations

The expected market for SUSs increased by 17.8% between 2016 and 2021, but it will continuously grow at a rate of nearly 15.5% over the forecast period, 2018 to 2023 [65]. This increment in SUSs systems has gained popularity because they have demonstrated that can provide an excellent option to adapt the production capacity to both, research and production demands [66]. This fact makes them a viable solution for mass production of different biological reagents in sensitive cells, antibodies or vaccine candidates to face pandemic-type situations such as COVID-19.

In the biopharmaceutical industry, the use of SUSs influences the production process with cost reduction, flexibility, time reduction when executing by eliminating additional steps and consequently with a reduction of cross contamination risks [67,68]. Also, its use significantly reduces process fluid waste, labor costs, and quality of in-situ validation requirements.

However, there are certain limitations in the use of these innovative SUSs mainly related to personnel training to deal with these new technologies, the challenges regarding the scaling up process due to the mechanical resistance of the bag's materials, as well as the optimization to determine appropriate working conditions at higher scales. Even so, the aforementioned limitations related to scaling up can be quickly overcome by the increasing automatization and the use of adequate biodegradable materials for their design.

Other possible limitations of SUSs are the risk of rupturing or leaking of disposable bags caused by their chemical, physical and biological properties; also, the migration of pollutants from the plastic material to the environment; and, the possible increase in operating costs due to the consumption of single-use materials such as bags, pipe filters, etc., resulting in high production of waste [69].

Despite the possible limitations, the use of SUSs is still a viable solution to the need for mass production of vaccines to face pandemic-

type situations such as COVID-19. This is due to the advantages that these systems have over conventional stainless-steel bioreactors in terms of production time and costs. Especially, the manageable parameters of the SUSs could be adjusted, benefiting the cultivation of mammalian and insect cells necessary for the production of vaccines and other applications such as clinical cell treatment, production of therapeutic proteins, among others.

Continuous research is still needed to establish a guide so that we could determine which production way is the most appropriate between conventional stainless-steel equipment and SUSs. The use of conventional or SUSs bioreactors will strongly depend on three main items: the purpose to be carried out, the bioprocess and the client specifications. These studies will be necessary to overcome as soon as possible any SUSs limitations in order to take advantage of its indisputable productivity advantages.

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Declarations

The authors declare that they have no conflicts of interest, that the work has been approved by the ethics committee responsible in the workplace, and do not declare means of financing of the work carried out.

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