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A Simulation Analysis of an Influenza Vaccine Production Plant in Areas of High Humanitarian Flow. A Preliminary Study for the Region of Norte de Santander (Colombia)

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Abstract: The production of vaccines of biological origin presents a tremendous challenge for researchers. In this context, animal cell cultures are an excellent alternative for the isolation and production of biologicals against several viruses, since they have an affinity with viruses and a great capacity for their replicability. Different variables have been studied to know the system's ideal parameters, allowing it to obtain profitable and competitive products. Consequently, this work focuses its efforts on evaluating an alternative for producing an anti-influenza biological from MDCK cells using SuperPro Designer v8.0 software. The process uses the DMEN culture medium supplemented with nutrients as raw material for cell development; the MDCK cells were obtained from a potential scale-up with a final working volume of 500 L, four days of residence time, inoculum volume of 10%, and continuous working mode with up to a total of 7400 h/Yr of work. The scheme has the necessary equipment for the vaccine's production, infection, and manufacture with yields of up to 416,698 units/h. In addition, it was estimated to be economically viable to produce recombinant vaccines with competitive prices of up to 0.31 USD/unit.

Keywords: modeling process; SuperPro Designer®; cell culture; public health; developing countries

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1. Introduction

Influenza is an acute viral infection that can cause symptoms ranging from mild to severe, including bronchitis, pneumonia, and death, especially in patients with risk factors [1]. Unlike in the United States, where there is one influenza season, in Colombia, it occurs in two peaks (March–June, and September–November) [2]. In 2019, prior to the COVID-19 emergency, approximately 6.7 million people suffered symptomatic influenza infection, with over twenty thousand hospitalizations in intensive care units; most of these cases were reported in the region of Norte de Santander [2]. The region of Norte de Santander is located on the northeast side of Colombia and shares a wide border with Venezuela. Due to the economic instability experienced in the last 10 years by the neighboring country, a substantial share of their citizens have migrated to Colombia and other Latin American countries using the control border located in the cities of Cucuta and Villa del Rosario [3].

In 2012, the Colombian Ministry of Health (Minsalud) launched the Ten-Year Public Health Plan (PDSP) 2012–2021 as a tool to strengthen access to health in an equitable and egalitarian way throughout the national territory [4]. According to the ministry, the

age-specific mortality rate for transmissible diseases in Norte de Santander is higher than the national median (46.8 and 34.50, respectively) [5]. For the region of Norte de Santander, respiratory infections accounted for 40% of care in all subgroups of the general population, also showing a trend towards an increase in cases [6]. The best way to control influenza outbreaks is through vaccination [7]; however, barriers to global implementation of vaccine production such as limited manufacturing, vaccine cost, and suboptimal efficacy [8] hampers the efforts to avoid unnecessary death. Despite the importance of vaccine production, no companies in Colombia are focused on producing influenza vaccines to the best of the author's knowledge.

SuperPro Designer® is a process simulator explicitly developed for the modeling, evaluation, and optimization of bioprocess unit operations [9], which can be used from conceptual design, process operation, and optimization [10], as well as process economics and waste stream characterization [11]. The present work carried out a preliminary study of an influenza vaccine production plant, using SuperPro Designer® v8.0 as an initial alternative for vaccine production in Colombia.

2. Materials and Methods

2.1. Process Description

The process of vaccine production using recombinant viruses and animal cell cultures consists of three critical steps that correspond to two industrial faces of the process (Upstream and Downstream). In the first step (Cell Propagation), MDCK cells (CCL-34TM) are produced in DMEM culture medium (mainly composed of amino acids, sodium pyruvate, vitamin B12, biotin, and ascorbic acid) [12]; this media is used with a wide variety of suspension cells and adherent mammalian cells including keratinocytes, primary rat astrocytes, and human melanoma cells [13].

Once the desired cell concentration is obtained, the cells are transferred to fermenters with higher working volumes (pre-inoculum). Once the optimal cell density is reached, the cells are transferred and incubated with the virus A/HK/403946/09 (H1N1) [14] (also known as virus infection and propagation) under specific culture conditions (37 ± 1 °C, pH of 7.4) [15]. The final stage (downstream) consists of the liberation, elicitation, purification, inactivation, formulation, and packaging of the vaccine (Figure 1).

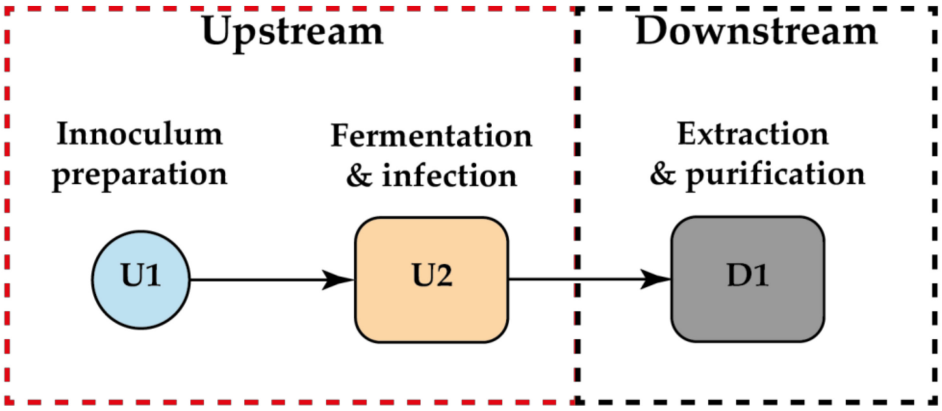

Figure 1. Process description of influenza vaccine production.

2.2. Plant Simulation

The influenza vaccine production plant was simulated using SuperPro Designer® software v8.0. (Intelligen, Inc., Scotch Plains, NJ, USA). This software allows us to compare different pre-treatment processes, production, consumption, and yields, making the process technically and economically feasible over time.

3. Results

Recombinant retroviruses and the massive culture of animal cell lines are fundamental techniques for large-scale biotechnological inputs to produce viral vaccines [16]. These vaccines are recombinant molecules obtained by expressing or replacing exogenous gene fragments in the host cell genome, which can be dispensed in the other cell sequences [17]. However, the production of retroviruses presents some difficulties, such as the need for a deep knowledge of the culture at different scales and the recovery technologies of these products; therein lies the importance of using simulation software to obtain production and cost estimates at an industrial level.

The use of simulators to model industrial processes is becoming more and more popular every day because it provides the opportunity to improve and reduce the time needed to generate different processes that integrate great applicability in different industrial branches [18]. These techniques are mainly based on the use and schematization of the process using mathematical tools and software, where all those independent and dependent variables (mass and energy balance) of the various biological and mechanical systems that make up the process are detailed to predict the natural behavior of the plant [19]. It also considers different aspects, including economic aspects such as construction, labor, equipment, waste, and total costs for the manufacturing and maintenance of the system. The operational, economic parameters of the system were classified in different sections: in the first one, we will find the total direct process costs (TIC), which include equipment, construction, maintenance, and labor (Total investment cost) (Equation (1)).

$$TIC = TPDC + TAC + CFD \quad (1)$$

According to Table 1, the main items that influence the total direct cost of the plant (TPDC) are equipment purchase, construction, and adaptation with percentages of up to 41, 11, and 10%, respectively, of the total TPDC. In contrast, the items related to the construction of the infrastructure is the element that has the most significant impact on the indirect costs of silver (TAC) (Table 2).

Table 1. Total direct plant cost (TPDC) (infrastructure costs).

Items	(USD $)
1. Equipment Purchase Cost	7,755,000.00
2. Installation	2,004,000.00
3. Process Piping	1,680,000.00
4. Instrumentation	1,921,000.00
5. Insulation	144,000.00
6. Electrical	480,000.00
7. Buildings	2,161,000.00
8. Yard Improvement	720,000.00
9. Auxiliary Facilities	1,921,000.00
Total	18,786,000.00

Table 2. Total Plant Indirect Cost (TAC).

Items	($)
10. Engineering	3,958,000.00
11. Construction	5,541,000.00
Total	9,499,000.00

Table 3 shows that the costs associated with the security of the project cover up to 10% of the total needed for the implementation of the system (CFD). Finally, Tables 4 and 5 show the operational costs of the equipment and raw materials of the system in question. The culture medium is a critical parameter within the production process, occupying up to 35% of raw materials.

Table 3. Contractor's Fee & Contingency (CFC).

Items	($)
12. Contractor's Fee	1,267,000.00
13. Contingency	2,533,000.00
CFC = 12 + 13	3,800,000.00

Table 4. Equipment's Purchase (Cost).

Operator	Labor (h/yr)	Labor (h/h)	Labor (h/kg MP)
V-101:P-I	11,314.29	1.43	N/A
ST-IOI:p-2	5657.14	0.71	N/A
50 L:R-3.	11,314.29	1.43	N/A
5L:R-2	11,312.29	1.43	N/A
500:R-4	11,314.29	1.43	N/A
O,5L:R-1	11,312.29	1.43	N/A
V-102:P-4	11,314.29	1.43	N/A
R-IOI:P-7	11,312.29	1.43	N/A
BM-IOI:P-6	5657.14	0.71	N/A
CF-IOI:P-9	1131.43	0.14	N/A
WSH-102:P-10	5657.14	0.71	N/A
MF-IOI:P-8	13,200.00	1.67	N/A
V-103:P-11	11,314.29	1.43	N/A
FL-IOI:P-12	565.71	0.07	N/A
RBS-IOI:P-13.	1346.00	0.17	N/A
Section Total	123,728.86	15.62	NIA
TOTAL	123,728.86	15.62	N/A

Table 5. Materials Cost.

Bulk Material	Unit Cost ($)	Annual Amount (kg)	Annual Cost ($)	%
CO_2	0.15	33,342.91	5001.44	0.00
DMEM	140.00	4,356,000.00	609,840,000.00	34.75
DPBS	2704.68	15,840.00	42,842,131.20	2.45
FCS	680.00	475,200.00	323,136,000.00	18.40
MDCK	320.00	39,600.00	12,672,000.00	0.71
Penicillin/strep	250.10	3,064,545.92	766,136,492.50	43.65
Potassium alum	0.18	616,713.92	111,008.51	0.02
VIRUS	1458.00	79.2	115,473.60	0.02
		TOTAL	976,095,107.25	100

3.1. Upstream

The upper part of Figure 2 shows the upstream scheme of the vaccine production system. The cells are strongly dependent on different factors such as nutrient concentration, gas injection, agitation, and other variables; therefore, the preparation and maintenance of the culture medium and culture system to be used are of great importance. In the present process, the DMEM culture medium is sterilized by dry steam (P2/ST-101) and supplemented by Fetal Bovine Serum (S-114). The scale-up process is increased up to a final working volume of 500 L (R4-/500) with a 10% (v/v) inoculum of MDCK cells (CCL-34TM) until reaching a cell density of 1.0×10^5 cells/mL, which allows decreasing the oncogenic capacity of the final product [20]. Likewise, the growth conditions were maintained at pH 7.4, 37 °C and 100% dissolved oxygen for the first 24 h, and then a reduction of up to 50% in oxygen saturation in the reactor [17]. The infection phase was performed considering the MOI multiplicity of infection factor of 2 [21], decreasing up to 13% of the cells not infected by the virus. Each reactor had a residence time of 4 days. Infection and virus adaptation was performed in stoichiometric fermentation with an infection rate of 87% of the produced cells. It is essential to highlight that the virus selected

for the present analysis has a high capacity for infection, reproduction, and adaptation in different types of cells such as respiratory, muscular, and kidney tissues, among others [14]. About 20 $\log^{10}$ genome copies/mL were obtained in the final fermentation process [22].

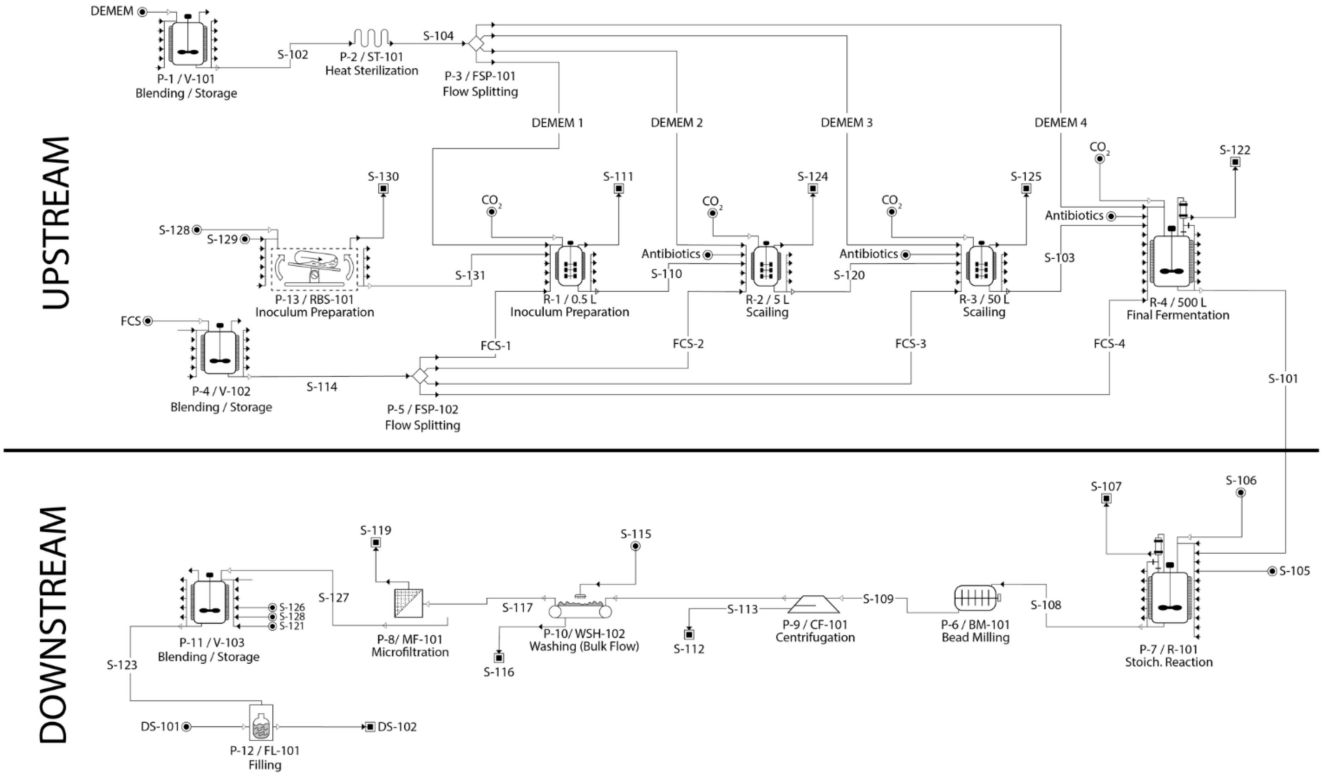

Figure 2. Flow diagram of the production, procurement, and purification process of influenza vaccine.

3.2. Downstream

The lower part of Figure 2 shows the downstream scheme of the vaccine production system. The process of obtaining the raw material begins with a bead mill (Bead Melling P-6/BM101) which accelerates cell lysis allowing the release of the viruses inside the cells; purification begins with precipitation using isopropanol at 50 °C at a ratio of 1:2, which is based on the differential solubility of the genetic material of the virion and is based on the decrease in the dielectric constant of the aqueous solvent, causing a decrease in the solubility of the DNA that allows the material to be separated and subsequently subjected to centrifugation (P-9/CF-101) to eliminate cellular debris.

Inactivation plays a vital role in this phase since it is only necessary to leave the genetic material inactive and the surface proteins unchanged so that it can be recognized by the immune system without affecting it, generating specialized antibodies for the destruction of its envelope. This allows the virion particles to be chemically inactivated with binary ethylamine (BEI) [23] and subsequently purified by salting out. These residual salts that allow the concentration of the vaccine are removed by microfiltration (P-8/MF-101) after washing to facilitate the permeation of the components and eliminate suspended solids, bacteria, proteins, or some dyes that may have been immersed in the inactivated virus [24]. Finally, the entire purified volume is mixed with adjuvants, Penicillin, Streptomycin, Amphotericin B, and salts as an antibiotic, antifungal, and preservative of the compound. Each vial is packed to a volume of 1 mL, containing 20 µg of inactivated influenza virus for a total yield of 3,083,569,629 units/yr.

Several authors have shown that the implementation of antigens using cell lines originating from different organs has facilitated the breakthrough in vaccine production since it allows the generation of biologics in a more accelerated way, with characteristics and public health standards that can compete with other vaccines [14,25]. However, according

to several authors, animal cellular tissues have a high immunogenicity and safety capacity for producing attenuated viruses [26–29].

4. Discussion

The cases of influenza in the region of Norte de Santander are of great importance for the epidemiological monitoring of respiratory diseases since its proximity to Venezuelan cities makes it an epidemiological focus that should be monitored. According to the Regional Institute of Health of Norte de Santander, the demand for health care services for communicable conditions ranked second in 2009–2018, with a slight upward trend at all times of the life course and in both genders. Likewise, within the subgroup of communicable conditions, respiratory infections accounted for 40% of care in all general population subgroups, showing an increasing trend in the frequency of these conditions [6]. The Expanded Program of Immunization implemented by the Colombian Ministry of health guarantees free access to influenza vaccines for infants only after completing the first year of life [30]. In the case of the adult population, free annual access to this biologic only benefits the population over 60 years of age, evidencing a failure in the coverage of access to this biologic in the general population, which is even more worrisome for the population of Norte de Santander, knowing the morbidity and mortality indicators previously mentioned [6]. The availability of interventions to meet this public health need, including annual access to influenza vaccine within the expanded program of immunizations, could prevent two-thirds of deaths from respiratory infections in children under five years of age, as well as reduce the demand for health care due to respiratory infections in all population groups in the Region of Norte de Santander [30].

Based on the modeling of the manufacturing process (Figure 2) and the information about the costs of setting up the system (Tables 1–5), it is possible to determine that the annual revenue is estimated at USD 1079 million with an opportunity cost of sale of an additional 10% of the production value. Additionally, based on the sale of USD 0.35 per unit, the payback period of the investment is 11.2 months, followed by annual liabilities of USD 103 million. The calculation of the gross cost of the product was obtained by dividing the number of doses obtained by the total cost of production, resulting in a cost of 0.31 USD/unit, the most relevant component being the operational cost of manufacturing. In work carried out by Aliya Mohamad Ros et al. [31], they evaluated the production of influenza vaccines using the Vero cell line. Their results identified that changes in cell line production kinetics affect the system's total cost. Likewise, and according to Farid [32], and Nestola et al. [33], the variables involved in the purification phase are the costliest (50%), followed by the production and maintenance phase (Tables 4 and 5). The processing, recovery, and purification of the different products associated with viral vaccines are critical processes for the stability of the process. Different techniques such as microfiltration have been considered for this stage; this technique can reduce costs by up to 70% compared to chromatography [24]. On the other hand, another possible way to reduce the size of the investment in fermentation equipment is the use of perfusion reactors; however, the difference lies in the initial investment costs since the production costs per unit are partially similar [34–36].

According to the results obtained in Tables 4 and 5, the manufacturing costs were about 30 times higher than the costs of plant establishment, mainly since the energy requirements for the physiological needs of the cell culture are high [37]. Likewise, in establishing the vaccine production system, the different costs related to vaccine supply and distribution were not considered, since the literature on the optimization of vaccine production in developing countries is quite scarce. However, according to Lee et al. [38], the costs of vaccine supply chains in developing countries are as much as 5 to 28 times lower than the total cost of production [39–41]. Likewise, the model presented here does not evaluate new emerging vaccine production technologies such as SARS-CoV2, since different kinetic parameters may change the model's performance. However, it presents a lean approach

to the industrial production system for vaccines of viral origin, estimating the different construction and production costs.

5. Conclusions

The production cost of the vaccine was estimated at 0.31 USD/unit, which is lower than the sales value of the producing pharmaceutical companies; however, this should be verified in a complete and detailed way for each operation, since the midstream sector, which includes transportation, storage, and commercialization of the product, was not considered. The implementation of SuperPro Designer® as a tool allowed considering the impact of the technical and economic analysis based on experimental studies, allowing the development of improvements in different production processes in comparison with alternative unitary operations, and the interaction of them with the other variables on a large scale. This also exposes that the raw material and size of cell production are a key part of the process.

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